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CONTENTS

NUMBER 1, FEBRUARY, 1938

Ménière's Disease. A Study Based on Examinations Made Before and After an Intracranial Division of the Vestibular Nerve. S. J. CROWE, M.D.....	1
Clinical and Experimental Results with Thorotrast. DAVID L. REEVES, M.D., AND RALPH M. STUCK, M.D.....	37
The Pathological Physiology of Chronic Cardiac Decompensation. M. D. ALTSCHULE, M.D.....	75

NUMBER 2, MAY, 1938

The Mammalian Blood, Platelet in Health and Disease. LEANDRO M. TOCANTINS.....	155
--	-----

NUMBER 3, SEPTEMBER, 1938

The Rheumatic Subcutaneous Nodules and Simulating Lesions. HARRY KEIL, M.D.....	261
---	-----

NUMBER 4, DECEMBER, 1938

The Coagulation of the Blood. P. NOLF.....	381
Plasma Fibrinogen Response in Man. Influence of the Nutritional State, Induced Hyperpyrexia, Infectious Disease and Liver Damage. THOMAS HALE HAM, M.D., AND FANNY C. CURTIS, A.B.....	413
Sedimentation Rate of Erythrocytes. Influence of Technical, Erythrocyte and Plasma Factors and Quantitative Comparison of Five Commonly Used Sedimentation Methods. THOMAS HALE HAM, M.D., AND FANNY C. CURTIS, A.B.....	447

MÉNIÈRE'S DISEASE

A STUDY BASED ON EXAMINATIONS MADE BEFORE AND AFTER AN INTRACRANIAL DIVISION OF THE VESTIBULAR NERVE¹

S. J. CROWE, M.D.

Baltimore, Maryland

This report is based on a study of 117 patients seen at the Johns Hopkins Hospital during the past nine years. The only type of therapy employed was surgical. In 23 the disease was relatively mild and no immediate operation was indicated. They have been followed, however, and re-examined from time to time. In the remaining 94 the attacks were extremely severe and disabling, so much so that having already tried one or more of the commonly employed therapeutic measures, such as dietary treatment and drugs, removal of teeth, tonsils, appendix or gall bladder, and in some cases extensive nasal sinus or pelvic operations, they came to the hospital to have their vestibular nerve divided. This operation is the only logical one for the relief of intractable vertigo, as was recognized by Charcot many years ago, but patients and their medical advisers are usually averse to a brain operation, and postpone it until the symptoms become unbearable. This is a mistaken idea. There is a great difference between the removal of a brain tumor and the operation for the cure of Ménière's disease. In one the intracranial pressure is increased while in the other there is no change whatever. Brain surgery has developed to the point where opening the skull to divide a nerve entails no more risk than opening the abdominal cavity.

In 90 per cent of our cases the disease was unilateral and hearing in the affected ear was markedly impaired. The operation on this group of patients was of two types. The entire auditory nerve was cut in the first 49 cases, curing the vertigo but destroying the remaining

¹ From the Otological Research Laboratory of the Johns Hopkins University. Aided by a grant from the Arthur B. Duell Fund for Research in Otology.

hearing. The necessity for preserving hearing, however bad, in the occasional case with bilateral Ménière's disease, or bilateral deafness due to other causes, led to the development of the second type of operation—division of the vestibular nerve alone. This was done to 45 patients in our group. To our surprise, an improvement in hearing on the operated side followed in 22 per cent of the cases. Division of the vestibular nerve is the essential part of the operation, and the ideal operation is one in which all vestibular fibers are divided and all cochlear fibers spared.

Our interest in following and talking with these patients from year to year, and retesting their auditory and vestibular apparatus is: to collect a group of facts as a basis for a better understanding of the disease, to learn the late results of the operation, and to try and deduce from these clinical data something regarding the nature and location of the lesion. Although Ménière's disease has been recognized for more than seventy years, no post-mortem examinations have ever demonstrated the lesion in a convincing manner. Until satisfactory necropsy studies have been made, our knowledge of this disease must rest on clinical observations.

Its diagnosis is not difficult. The characteristic symptoms are: the sudden onset of vertigo with a sensation of the rapid spinning of the patient himself, or of surrounding objects; nausea and vomiting; deafness of the inner ear type, which is usually unilateral and grows progressively worse, and tinnitus, which is commonly referred to the deaf ear. The attacks come at irregular intervals, and in this group of patients they recurred with increasing frequency and severity. Aside from the tinnitus and deafness, the patient feels perfectly well between attacks, with no trace of dizziness, unsteadiness or incoordination of muscular movements. The vertigo may begin without warning, or may be preceded by increasing tinnitus or other aura. The attacks come during sleep, while at work, walking, or sitting quietly reading, and may be so severe that the patient is thrown to the floor. Fifteen of 117 patients had occasional unconsciousness in the attacks, but never any convulsive movements; and 48 had remissions, or intervals between their attacks, varying from one or two months to twelve years.

Many of these symptoms have been known for more than a century,

but it was Ménière in 1861, who first suspected that they were due to a disorder of the inner ear. The current belief at that time was that they resulted from cerebral congestion or apoplexy. Indeed, Ménière thought the disease he described was due to intralabyrinthine hemorrhage. He reached this conclusion after observing a patient with violent vertigo, nystagmus, nausea and vomiting, and at autopsy finding a bloody fluid in the semicircular canals. From his description of the symptoms, the rapidity with which they advanced, and the associated fever, it is probable that his case had an acute purulent labyrinthitis and not a hemorrhage. As we read the clinical histories and talk with Ménière patients, it is impossible to believe that their symptoms result from intralabyrinthine hemorrhages. Although

TABLE 1

Showing that 110 of 138 patients were between the ages of 30 and 60 years at the onset of their first symptom of Ménière's disease

AGE OF PATIENTS AT ONSET	NUMBER OF PATIENTS
10 to 19 years	3
20 to 29 years	13
30 to 39 years	33
40 to 49 years	43
50 to 59 years	34
60 to 69 years	12
Total	138

Ménière's disease is most common in the fourth, fifth and sixth decades (table 1), the patients as a group are in unusually good physical condition. Very rarely do they have nephritis, cardio-vascular disease, a blood dyscrasia or syphilis. The average blood pressure in an unselected group of 68 of our cases was 137/83. The systolic pressure was above 150 in eleven, and below 100 in none. The diastolic pressure was above 100 in seven, and below 60 in only two patients. It can also be stated definitely that otitis media, sinusitis, tonsillitis, asthma, hay fever and disorders of the thyroid gland, are not the cause of this malady, for these diseases were rarely present in this group.

Charcot in 1870, demonstrated cases of Ménière's disease at his clinics, and observed that in some patients the vertigo attacks ceased

when deafness became complete. This led him to suggest that the cure could be hastened by an intracranial division of the auditory nerve. It was long before the era of brain surgery, however, and although various attempts to cut the nerve or destroy the labyrinth have since been made, the report of Dandy (1) in 1928, was the first to present favorable results on a large series of cases. He has now operated on more than 200 patients with Ménière's disease without a fatality, and with uniformly good results.

In addition to the history, general physical and neurological examination, all our 117 patients have had two or more otoscopic, vestibular, tuning fork and audiometer tests. The threshold acuity for air conduction throughout the entire range of hearing, and the ability of the patient to understand and repeat correctly a series of numbers, recorded by male and female voices on a phonographic disc, were all measured with the audiometer. The hearing by bone conduction was tested with a 512 d. v. tuning fork. All tests were made in the sound-proof room of the Otological Laboratory, which eliminates outside noise and adds to the accuracy of the record. If the audiometer and its receivers are properly constructed and calibrated, this instrument affords the only objective method, and therefore the most accurate means of testing the hearing. The audiometer chart is a record of the number of decibels of electrical energy, necessary to produce the faintest sound that can be heard by the patient. The audiometer chart alone, however, does not provide sufficient data on which to base a diagnosis of the type of deafness: it must always be checked and supplemented with tuning fork and voice tests, an examination of the tympanic membrane, nasopharynx, eustachian tubes and the upper air passages. After the hearing tests are completed, the degree of deafness is estimated by comparing the results with the hearing of normal persons of approximately the same age (2).

The importance of the principle of masking must be emphasized here, because it is an essential part of the technique for the examination of patients with Ménière's disease, and for all other types of deafness in which the inner ear is involved. Masking removes the subjective element in the hearing test. The noise reduces the hearing acuity somewhat, but if used at the same intensity first on one side and then on the other, it in no way interferes with a comparison of

the two ears. Barany devised many years ago a mechanism operated by clockwork to produce the masking sound; others use a jet of compressed air in the external auditory canal, but the most satisfactory instrument for this purpose is an electrically operated buzzer, with a rheostat to control the intensity of the sound (3). The intensity used for masking is recorded on the audiometer chart and is varied according to the degree of deafness in the opposite ear: thus, the conditions at the first test may be duplicated at subsequent examinations.

The sensory end-organs in the mammalian inner ear are six in number: a crista in the ampullated end of each of the three semi-

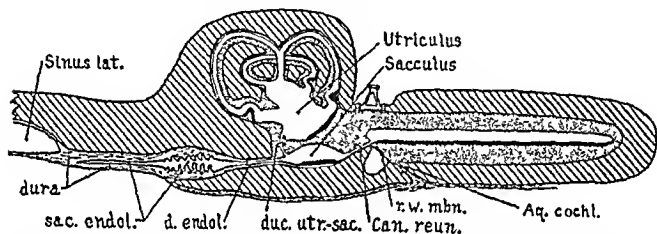


FIG. 1. In this schematic representation of the inner ear, the cochlea is shown a "unrolled"; bone, except for the stapes, is indicated by oblique hatching, perilymph by stippling and the six sensory end-organs (cristae, maculae and Corti's organ) by thick solid black. The cavity of the membranous labyrinth, which is filled with endolymph, is represented by the open spaces. Through the canalis reuniens (*can. reun.*) and ductus utriculo-sacculus (*duc. utr-sac.*) there is free communication of the endolymph in the larger spaces of the membranous labyrinth. The other narrow part of the membranous labyrinth, the ductus endolymphaticus (*d. endol.*), conveys endolymph to its region of outflow or resorption in the sacculus endolymphaticus (*sac. endol.*). The cochlear aqueduct (*aq. cochl.*) connects the basal end of the scala tympani, near the round window membrane (*r. w. mbn.*), with the subarachnoidal space.

circular canals, a macula in the utriculus and in the sacculus, and the organ of Corti in the cochlea. The nerves from the three cristae and the two maculae join in the internal auditory canal to form the vestibular part of the eighth cranial nerve. The fibers from the organ of Corti emerge through about 150 separate foramina in the base of the modiolus to form the auditory part of the eighth nerve. These two nerves come together in the medial end of the internal auditory canal, and for a few millimeters they have a common sheath. This explains the accidental cutting of cochlear fibers when the vestibular nerve is divided at operation. The trunk of the eighth

nerve breaks up into several bundles (rootlets) near the brain stem, and the cochlear and vestibular portions pass by distinctly separate routes to their respective nuclei of reception in the medulla oblongata. At this point the inferior cerebellar peduncle separates the ventral and dorsal cochlear nuclei from the vestibular nuclei, Deiters' and Schwalbe's, and nowhere do the central pathways of these two nerves come in contact. A single lesion in the brain stem or cerebrum could not cause the auditory and vestibular symptoms of Ménière's disease without involving other structures. Anatomically there are only two areas in which a single lesion could cause these symptoms: in that part of the nerve trunk where the cochlear and vestibular nerves are contained in one sheath, or in the inner ear. Although Corti's organ and the vestibular end-organs are widely separated, they have one thing in common—the endolymph (fig. 1). This fluid fills the membranous labyrinth, and any change in its chemical or physical properties could affect both the cochlear and vestibular end-organs.

VERTIGO

The chief symptom of Ménière's disease is vertigo. Stimulation of the vestibular end organs in any of the functional tests, produces sensations very like those experienced in Ménière's disease. The severity of the symptoms depends on the stimulus. They may be mild and transient, or severe and prolonged, and the variations are in direct proportion to the strength and duration of the stimulus. Patients with Ménière's disease dread an examination that makes them dizzy, and for this reason the caloric instead of the turning test is used. The external auditory canal is irrigated with cold (64°F.) water, just enough to produce a minimal nystagmus reaction.

The vestibular responses to the caloric test have been tabulated under three headings: normal, subnormal and no reaction. By "normal" we mean that the patient has nystagmus, a sensation of dizziness, and muscular incoördination, which manifests itself in a staggering gait or falling. Excessive stimulation accentuates these symptoms, and in addition causes sweating, nausea and vomiting, and must be avoided. The term "subnormal" means that three or four times the usual amount of water is required to produce a minimal reaction, and "no reaction" that even with a much greater stimulus,

there is no response. Of 117 patients with Ménière's disease, the caloric test was "normal" on both sides in 41 or 35 per cent, and "subnormal," only on the affected side, in 22 or 19 per cent. These figures indicate that the vertigo in 54 per cent of the entire group is due to an irritative and not to a destructive lesion. There was "no reaction" on the affected side in 34 patients or 29 per cent. We believe that the end organs in this latter group were more damaged, possibly by edema, than were those in the "subnormal" group; and therefore failed to react to the relatively weak stimulus of the caloric test, but did react to the stronger irritant of the disease. Otherwise these patients could not have had vertigo. For various reasons no vestibular tests were made in 20 patients.

Vertigo, such as occurs in Ménière's disease, is not possible after destruction of the static labyrinth or section of the vestibular nerve, and total loss of reaction to the caloric or tuning test is usually interpreted as evidence of a destructive lesion. But these 34 patients, with no vestibular response whatever, and whose chief complaint was vertigo, were all cured by cutting the vestibular nerve. This is proof that their vertigo attacks were due to irritation on the side with a negative caloric test, and that vestibular tests are unreliable as diagnostic aids. Additional evidence of the inconsistency of these tests, is furnished by the fact that in four patients there was "no reaction" to caloric stimulation at the first examination, while a "subnormal," but definite reaction, was obtained at subsequent examinations. As the technique of the tests was the same, local conditions in the ear must have changed. In two other patients, there was "no response" to caloric stimulation, but a "normal" response to the turning test, which affords a much more powerful stimulus. If the symptoms of Ménière's disease were caused by a lesion, which destroyed the labyrinth or the nerves, they would be self limited; but many of our patients have had tinnitus, fluctuating hearing, or attacks of vertigo for fifteen or twenty years. Only an irritative lesion could cause these symptoms over such a long period.

It is not practical to use a stronger vestibular stimulus when examining patients with Ménière's disease, and therefore a decision as to which ear is causing the symptoms depends on the history of the attacks, the location of the tinnitus, and the demonstration of an

inner ear type of deafness. When these symptoms are bilateral and equal, it must be assumed that both ears are responsible. Under these conditions, the nerve is cut on one side and, if the vertigo continues, on the other.

This brings us to a consideration of the effects of bilateral section of the vestibular nerves. In 1937, Dandy (4) reported his post-operative observations on ten cases. The spontaneous attacks and the dizziness on turning the head always disappear, but these patients have some disturbance, probably permanent, connected with the eyes (5). Walking in the dark or with eyes closed causes staggering and a feeling of insecurity. At other times the gait is normal. When doing any work that requires movement of the head, such as sawing a board along a straight line, the vision becomes blurred. When walking, they complain of inability to see clearly, apparently due to difficulty in focusing on objects when in motion. The vision is normal when sitting quietly in a chair or standing still. These visual symptoms are usually present for a short time after section of one vestibular nerve, but are so disturbing after bilateral section, that division of the second vestibular nerve should be postponed until it is known that unilateral division has not been effective.

When a patient is perfectly well between attacks of vertigo, or on the other hand, has constant dizziness with acute exacerbations in which there is a sensation of objects spinning around him, or of his own body whirling, the trouble is due to Ménière's disease. Acoustic tumor, however, must be thought of in this connection. Dandy (6) has reported such cases, but in all of them the symptoms of increased intracranial pressure and involvement of other nerves, were superimposed on the usual symptoms of Ménière's disease, and became evident sooner or later. Cases with these dual symptoms are exceptional, and not one in this group of 117 patients had any evidence of increased intracranial pressure or new growth.

A history of "whirling of surrounding objects" during an attack is most important in differentiating between the dizziness of Ménière's disease and that due to other causes. However, the direction in which objects seem to whirl is of little value in determining which ear is causing the trouble. The following analysis of the records of 37 patients shows plainly how irregular is the relation of these two

factors, and that the direction in which objects whirl during an attack can have no diagnostic significance.

	<i>Number of cases</i>
Deafness on the left: objects whirl toward the right.	16
Deafness on the left: objects whirl toward the left.	6
Deafness on the right: objects whirl toward the left.	7
Deafness on the right: objects whirl toward the right.	8

Identical results can be obtained in normal persons, by irrigating the external auditory canal on one side with cold, and then with hot water, or by whirling in a chair, first in one and then the other direction. The explanation of such opposite results in vestibular tests is the reversal of the flow of endolymph in the semicircular canals. The similarity of the symptoms of Ménière's disease to those produced by artificial stimulation of the labyrinth, furnishes additional evidence that this disease is due to a disorder in the static labyrinth, and not in the nerve or central vestibular pathways.

Other symptoms usually but not invariably present in Ménière's disease, such as tinnitus, fluctuating hearing and progressive deafness, are evidences of irritation, or permanent damage to the auditory apparatus. The end organs of the vestibular and cochlear nerve are surrounded by perilymph and endolymph, and the intra-vestibular and intra-cochlear fluids freely communicate. The perilymph is cerebro-spinal fluid, and through the cochlear aqueduct the perilymphatic spaces in the cochlea communicate freely with the sub-arachnoid space of the posterior fossa. That changes in pressure of this fluid can have nothing to do with the symptoms of Ménière's disease is proved by their absence in hydrocephalus, and in the more acute increase of intracranial pressure in brain tumors. The perilymph is nowhere in direct contact with the cochlear or vestibular end organs. The endolymph is secreted by a kind of choroid plexus within the cochlea, and is in direct contact with the organ of Corti and the vestibular end organs. It is in a closed system and has no connection with the perilymph or the cerebro-spinal fluid. The special sense cells, in which the cochlear and vestibular nerves end, are bathed in endolymph, which is thought to convey oxygen to the cells of Corti's organ, and to carry away the waste products of metabo-

lism. It is conceivable, therefore, that chemical, circulatory, or pressure changes in the endolymph could produce the auditory symptoms by irritation or injury to the cells of the organ of Corti; and the vestibular symptoms, by a cumulative effect of the irritant on the end organs of the vestibular nerve. These intralabyrinthine fluids form the only communicating link between the auditory and vestibular structures in the temporal bone.

It has been suggested that "toxic neuritis" of the eighth nerve is responsible for the symptoms of Ménière's disease. Tinnitus may be the only symptom for months or years, the deafness may disappear after an attack of vertigo, or become progressively worse, and the attacks of vertigo may occur frequently or at long intervals. These are not the symptoms one would expect from a neuritis. Theoretically, the mode of onset and later symptoms support the neuritis theory, but changes in the nerve, sufficient to cause these symptoms, should be evident in histologic sections. They are not. In two cases, a piece of the auditory nerve was removed at operation, and failed to show any abnormality. Furstenberg (7) also examined a piece of the nerve, removed at operation in eleven cases, and came to the same conclusion. An important argument against this neuritis theory is the notable absence of infections in the upper air passages, and of symptoms in the eyes, joints or other parts of the body, referable to focal infections.

Another explanation of the cause of Ménière's disease is based on the occasional finding, at operation, of a branch of one of the cerebellar arteries lying on the eighth nerve, or looped around it (6). A sclerosed artery in this location might reduce the blood supply to the inner ear, or directly injure the nerve; but neither the sclerosis, nor the nerve injury have been demonstrated histologically. Evidence of arteriosclerosis in the brain or eyes is rare in this group. Although 110 of 138 patients were between the ages of 30 and 60 years, their average blood pressure was normal (137/83). A comparison of two groups, with and without these abnormal vessels on the auditory nerve, shows that so far as the history and symptoms are concerned, they differ in no important respect.

The vertigo and deafness could arise from a lesion in the nuclei or pathways in the brain. There are objections to this theory also.

The auditory and vestibular pathways diverge soon after entering the brain stem, and to explain the symptoms of Ménière's disease one must postulate two independent lesions, or one lesion extensive enough to involve both pathways, but at the same time sparing all other nerves in this region. In the majority of our cases no neurological lesions, other than those of the eighth nerve, were found. It should be noted, however, that in five of a total of 100, the patients complained of diplopia with or following the attacks of vertigo; but we were never able to demonstrate a lesion of the third, fourth or sixth nerves in these cases. One patient had numbness of the right leg following his attacks; the right auditory nerve was cut, all of his symptoms disappeared, and when last seen three years later he had had no recurrence. Another had numbness of both arm and leg on the right side; his trouble was also in the right ear. The vestibular fibers alone were divided, and a year later he reported no recurrence of any of his previous symptoms. Fifteen patients were unconscious in some of their attacks, but in no case did this symptom recur after operation. Their loss of consciousness, or fainting, was probably due to a fall in blood pressure. The vertigo, tinnitus and deafness of Ménière's disease, can be explained by a labyrinthine disorder, but the more general neurological symptoms in these 21 patients could not be due directly to any lesion in the inner ear. Headache preceding the vertigo was present in twenty seven of the 117 cases in this group: it was on both sides in nine, and limited to the side with the affected ear in fifteen; while three patients had only a feeling of fullness "like water" in the affected ear.

Tinnitus and vertigo may originate in a totally deaf ear. In the following case the absence of response to audiometer, tuning fork, voice and vestibular tests, indicated a total loss of function in the labyrinth on the left side. The results of section of the left auditory nerve prove that this conclusion was incorrect, and that the symptoms did come from the inner ear on this side, and not from a lesion in the brain.

E. K. (66365). A man, aged 25 years, since having bilateral otitis media at the age of seven has been totally deaf in the left ear, and partially deaf in the right. His first attack of vertigo came suddenly and without warning, two months before admission to the hospital. There was a

sensation of objects whirling about him, he fell to the floor, and for a time was unconscious. Tinnitus appeared for the first time after this attack, and was localized in the left or totally deaf ear. The Wassermann test was negative. His blood pressure was 140/85, and the physical and neurological examinations were negative, with the exception of a history of diplopia with the vertigo attacks. The tympanic membranes were intact but markedly retracted. Bone conduction was nil on both sides. All tests showed total deafness on the left, when the right ear was masked. The entire eighth nerve on the left was cut. In a letter written nearly two years after the operation, the patient says that he is well, has gained thirty pounds, works regularly, and has had no vertigo or tinnitus since leaving the hospital.

Discussion. The left side was chosen for operation because the tinnitus was on the left, but largely for fear of endangering the slight hearing which he still had on the right. According to functional tests, both the vestibular and cochlear nerves on the left were dead, but in this, as in similar cases, section of the nerve resulted in a cure of the vertigo. It is difficult to explain the immediate and complete cessation of the vertigo after cutting the left auditory nerve, if, as the functional tests indicated, the labyrinth on this side was totally destroyed. The symptoms must have originated from irritation in the left labyrinth, and not from the central auditory and vestibular pathways.

We firmly believe that the vertigo and deafness of Ménière's disease are always due to stimulation of, and degenerative changes in, the peripheral auditory and vestibular structures; that it is impossible to have vertigo after section of the vestibular nerve, and that the cure of the disease is due to this fact. If this is true, we must conclude that functional vestibular tests are not always reliable or accurate diagnostic guides.

A typical case history, which illustrates the progression of symptoms in Ménière's disease, is that of J. R.:

J. R. (No. 68947), a laborer, aged 53 years, first came to the out-patient department in April, 1932. At this time his complaints were: tinnitus in the left ear: "strange sensations" in the left side of his head, and slight deafness in the left ear. The tinnitus annoyed him more than the other symptoms: it was described as a loud, high pitched, ringing noise. His trouble dated from a mild and transient otitis media, on the left side, six

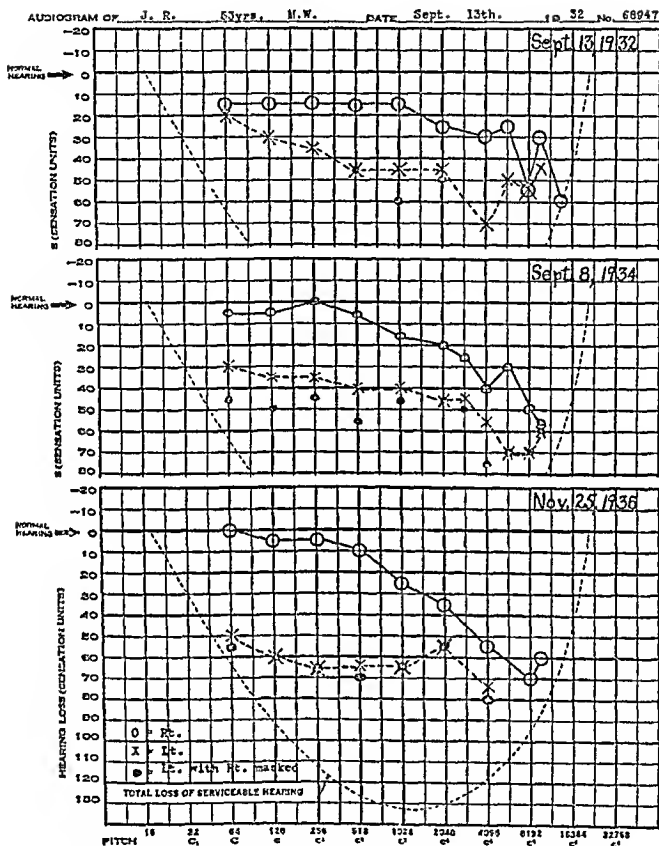


FIG. 2. These three audiograms show a progressive loss of hearing in the left ear. Note that the high tones were first affected. The examination in November, 1936, was made six months after the intracranial division of the vestibular nerve on the left. He has had no vestibular symptoms of Ménière's disease since the operation, but the deafness continues to progress. Tinnitus was unchanged by the operation.

months before. The ear infection healed within two weeks, but the other symptoms persisted, and the tinnitus grew louder. When questioned, he stated that he "occasionally felt a little dizzy when in a warm room," but at this time he had never had an attack of vertigo. Examination in the medical department disclosed nothing abnormal, aside from moderate sclerosis of the peripheral arteries. His blood pressure was 138/86, and the Wassermann test was negative. The tympanic membranes were intact, and no focal infection was found.

Tuning fork tests and audiometer record (fig. 2), showed an inner ear type of deafness on the left. The Weber test was referred to the right or good ear. Air conduction was better than bone conduction on both sides, and the bone conduction time was shortened on the left, as compared with the right. No diagnosis was made, and the patient was requested to return for additional study.

His next visit was two years later, and his complaints were: violent attacks of vertigo, loud tinnitus in the left ear, progressive deafness on the left (fig. 2), and headache also on the left side. Turning his head quickly made him dizzy, but did not bring on an attack of vertigo. These came suddenly with no warning. Several times he fell while at work, or when walking on the street. The attacks often lasted for twenty-four hours; he was never unconscious in an attack. Section of the left vestibular nerve was advised, but he refused, because the vertigo attacks were infrequent, and he was afraid he would lose his job.

He was next seen in May, 1936, four years after his first examination. The vertigo attacks were then so frequent and severe that he could not work. The vestibular part of the left auditory nerve was cut.

When last examined, six months after the operation, he was working regularly. He had had no vertigo or dizziness with head movements since the operation. The headache disappeared soon after the operation, and had not recurred. The deafness continues to progress. The tinnitus was unchanged.

Discussion. The headache was due to the condition in the left ear, as proved by its disappearance after the operation. When the dura was opened, nothing abnormal was seen in the cerebello-pontine angle, but the subsequent history of this and many other patients suggests that in addition to cutting the nerve the operation did something to relieve pressure, possibly in the labyrinth. It is conceivable that increased intralabyrinthine (endolymph) pressure caused first the tinnitus, then the loss for high tones, and later a progressive loss for

all tones (fig. 2) as the damage to Corti's organ became more extensive. As the endolymph disorder in the cochlea increased, the static labyrinth became involved, and the severe vertigo attacks began. The attacks became more frequent and severe, the patient was unable to work, and relief was necessary. We have followed and made several examinations on 94 such patients, some for as long as nine years after operation, and the results afford convincing proof that attacks of vertigo are no longer possible after the vestibular nerve on the affected side has been cut. We have no proof to support the theory that an endolymph disorder is the cause. The post-operative results support equally well the belief that changes in the cochlear nerve may cause the symptoms of Ménière's disease.

Some patients with Ménière's disease have periods of constant dizziness, when they are confined to bed for weeks or months. Any movement of the head usually increases the dizziness, nausea and vomiting. Superimposed on this constant state of subacute dizziness are sudden, violent attacks of vertigo, which are so characteristic of this disease. This condition was present in twenty-three of the 100 histories reviewed for this point.

The history of R. C. (No. 52394), a white woman of 40 years, illustrates the gradual development of the disease over a period of seventeen years, long periods of remission, the effect of certain positions and movements of the head and body on the dizziness, and a long period of constant dizziness. The first symptom was the sudden onset of tinnitus in her left ear seventeen years ago. Several months later she had a series of attacks of vertigo with nausea and vomiting. Then for two years all of these symptoms disappeared. For no apparent reason they returned, each attack lasting for several hours. A second remission lasted for three years, and the vertigo again recurred. About seven years after the onset of the disease she noticed for the first time impaired hearing on the left, and during the past ten years the tinnitus and deafness on the left side have been present constantly.

During the past two years, the vertigo attacks have become more frequent and severe than ever before, and for the three months preceding admission to the hospital she was confined to bed. When attempting to get up she has frequently fallen to the left, and objects in the room seemed to whirl toward the left. She was unable to lie flat on her back or on the

left side, but was more comfortable on her right side. The tympanic membranes were normal. No infection was found in the upper air passages.

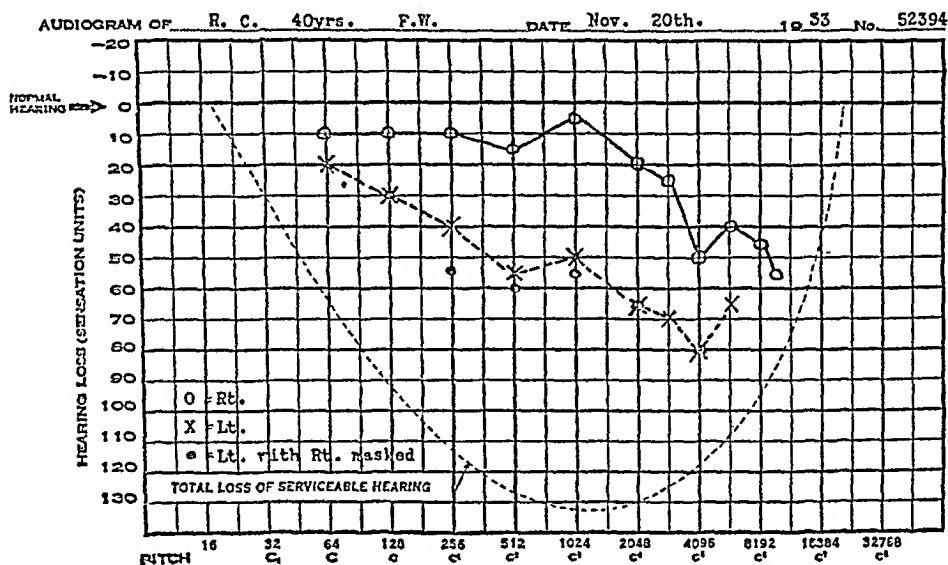


FIG. 3. Note that the hearing is more impaired for high than for low tones, but all tones from 64 to 5793 d. v. are heard.

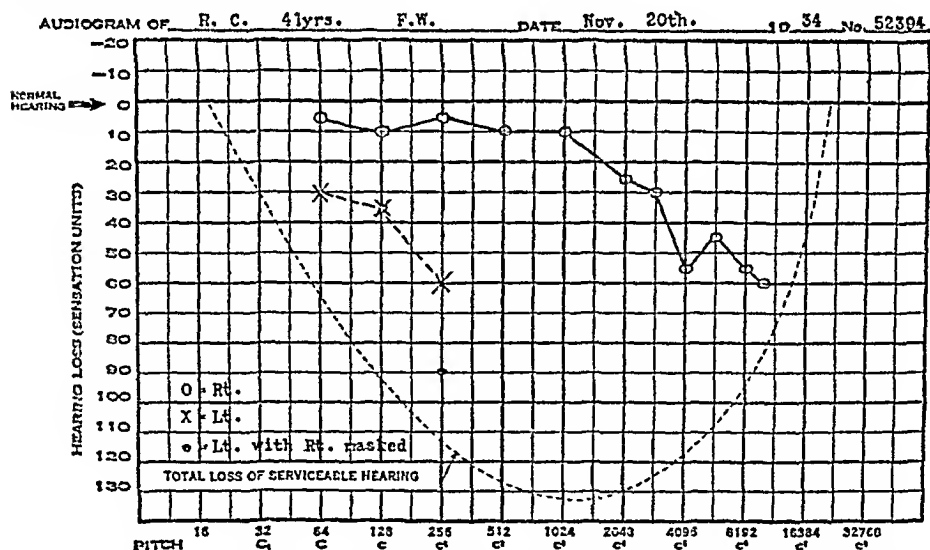


FIG. 4. When the vestibular nerve was cut some of the cochlear fibres were also divided. The result is a loss of four and one-half octaves, as shown by comparing figures 4 and 5.

The general physical and neurological examinations were negative. Air conduction was better than bone conduction on both sides. Bone conduc-

tion time was sixteen seconds on the right and nil on the left for the 512 d. v. fork. The audiometer test shows some impairment for the high tones on the right, but very marked loss on the left (fig. 3). The vestibular caloric response was nil on the left, and subnormal on the right.

Discussion. At operation the vestibular and cochlear nerves were so closely bound together, that in order to be sure of cutting all vestibular fibers, it was necessary to divide most of the cochlear nerve. The result shown in figure 4 is of interest from the point of view of the localization of tones in the cochlea. Repeated tests with the opposite ear masked, demonstrate that she is totally deaf on the left for all but the three lower octaves, while before operation she heard all tones from 64 to 5793 d. v. The patient is very intelligent, and we feel sure that these tests are accurate. A degenerative change in the peripheral neurone of the cochlear nerve always impairs the hearing for the high frequencies first. This is true in the early stages of an acoustic tumor, and in Ménière's disease. It is always the higher tones that are lost after a partial section of the cochlear nerve. These findings suggest that localized areas of Corti's organ and only certain fibers in the cochlear nerve transmit high tones; that low tones are not localized in the cochlea and are transmitted by any undamaged fibers in the cochlear nerve.

This patient was much improved by the operation. The tinnitus is still present in the left ear, but is much softer and lower in pitch.

AURA

G. H. (No. 64656), a white man, aged 46 years, came to the hospital in September, 1935, complaining of tinnitus and progressive loss of hearing in the right ear for fifteen years, and vertigo for twelve years. With the vertigo attacks he had nausea, vomiting, staggering, a sensation of objects whirling, and he fell toward the right. For many years the attacks were infrequent, but for three months before admission they had been frequent and severe, he had been unable to work, and had lost 35 pounds.

The aura in this case was tinnitus, which became much louder just before an attack until "the sound seemed to shoot out into space." This was always followed by severe vertigo. For one year after section of the vestibular nerve he had no tinnitus, but it then returned and as formerly was at times much more pronounced. Since the operation he has had no vertigo.

Discussion. From a total of 100 cases, thirteen gave a history of warning symptoms preceding the vertigo. These were as follows:

	<i>Number of cases</i>
Increased tinnitus, sometimes in the head, usually in the bad ear.....	6
A feeling of fullness, sometimes in the head, usually in the bad ear.....	2
Faintness and nausea	2
Increased deafness and tinnitus, both of which improve following the vertigo attacks.....	1
A burning sensation in the throat, which improves following the vertigo attacks.....	1
Tingling and numbness of the fingers of both hands, both of which improve following the vertigo attacks.....	1

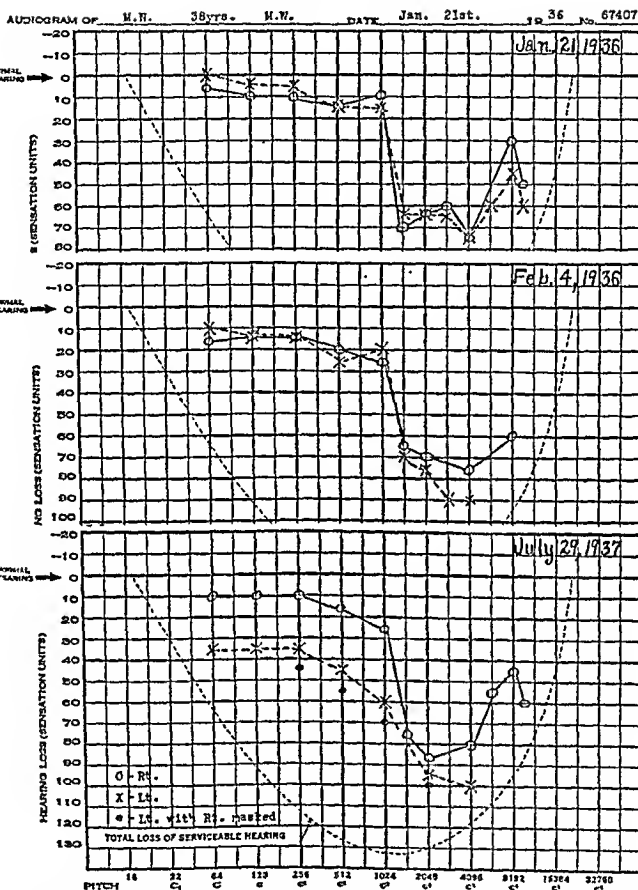
These premonitory symptoms occurred regularly, and if we except the last two cases, they might all have arisen from irritation of the auditory and vestibular structures in the temporal bone. Irritation in the central pathways could hardly have been responsible for this particular group of symptoms.

LOSS OF CONSCIOUSNESS

There was a history of occasional loss of consciousness in the attacks of vertigo in fifteen or 12.8 per cent of 117 patients. This symptom was never observed while in the hospital, but the description given in the histories indicates that these patients actually were unconscious for periods varying from a few minutes to an hour. There were no convulsive movements or other evidence of epilepsy. The blood pressure was not elevated, only four had headache with the attacks, the neurological examination was negative with the exception of the eighth nerve, and at operation nothing was found peculiar to this group.

These fifteen patients were again examined from one to seven years later, and neither the vertigo nor the attacks of unconsciousness had recurred. This means that the latter were in some way associated with the vertigo, and probably due to a sudden fall in blood pressure.

The following case history illustrates loss of consciousness and severe vertigo cured by operation. Continued headache and progressive deafness on the operated side, however, indicates that the disorder in the inner ear is still active.



M. N. (No. 67407), a laborer, aged 38 years, had his first attack of vertigo a year before admission to the hospital. It came on suddenly with no warning symptoms. He had a sensation of objects spinning from right to left, fell, and was unconscious for about fifteen minutes. When he recovered, he noticed for the first time a noise in his left ear "like escaping steam." The vertigo attacks recurred with increasing severity every two or three weeks for several months, then became so frequent that he was totally incapacitated. He was unconscious only in the severest attacks. The physical and neurological examinations were negative. The Wassermann was negative and the blood pressure normal (125/85). The accessory nasal sinuses, eustachian tubes and tympanic membranes were normal. He was not deaf, and heard conversation on both sides equally well. Air conduction was better than bone conduction in both ears, and the bone conduction time for the 512 d. v. fork was the same on the two sides. The audiometer test shows a marked bilateral impairment for the higher tones² (fig. 5A). The vestibular caloric test was normal on the right: prolonged stimulation on the left caused nausea but no nystagmus, falling or pointing reaction. The vestibular nerve on the left was cut. This side was selected because the tinnitus was in this ear only.

Two weeks after operation the hearing on the two sides was the same except for a loss of some higher tones, due to the accidental division of a few cochlear fibers when the vestibular nerve was cut (fig. 5B).

When the patient was last examined one and a half years after operation, he was working regularly, and had had no recurrence of the vertigo or attacks of unconsciousness. The tinnitus in the left, or operated ear, was the same as before operation, but recently he has had increasing deafness in this ear. The audiogram shows how much the deafness has progressed (fig. 5C). He also has headache on the left side, but the neurological and physical examination is still negative. The disorder in his inner ear that caused the attacks of vertigo is still active, and accounts for his progressive deafness.

REMISSIONS

Spontaneous remissions of vertigo, and sometimes of tinnitus and deafness, are some of the manifestations of this disease that we find hard to explain. Remissions are periods of freedom from vertigo or

² The histology and pathology of deafness for high tones in patients with no vertigo were described from this laboratory in 1934. Crowe, S. J., Guild, S. R., and Polvogt, L. M.: Observations on the pathology of high-tone deafness. Bulletin of the Johns Hopkins Hospital, Vol. 54, pp. 315-379, 1934.

dizziness, and sometimes from deafness and tinnitus also Of 117 of our patients 48 had intervals between attacks that varied from one or two months up to twelve years

<i>Length of remission</i>	<i>Number of cases</i>
12 years	1
9 years	1
7 years	1
5 years	2
4 years	3
3 years	3
2 years	3
1 year	8
4 to 8 months	21
1 to 2 months	5
	<hr/> 48

These patients all had at the beginning of their illness one or more severe attacks, followed by intervals during which they thought the disease was cured Some of these remissions followed operation or treatment, such as the diet of Furstenberg or Mygind, the extraction of teeth, sinus operation, removal of tonsils and adenoids, or eustachian tube inflations, but for the majority there was no apparent cause As a rule the period between attacks became shorter, and the vertigo was more severe with each recurrence It was for this reason that the forty-eight patients listed above came to the hospital for division of their vestibular nerve There was nothing in their history to suggest a cause for the free periods The pre-operative examinations, the findings at operation and subsequent examinations differed in no important respect from those who did not experience remissions Spontaneous remissions do occur in Ménière's disease, and we should be more critical of the multitude of therapeutic measures advanced for its cure

The case of C L R (No 47262) illustrates the onset of the disease in a child aged 14 years, a remission for 12 years, then a recurrence of vertigo which was so disabling that operation was necessary When first seen in January, 1933, this patient was 26 years of age and had just graduated from the medical school When 14 years of age he began to have violent attacks of vertigo with nausea, vomiting, tinnitus and deafness in the right ear The vertigo attacks lasted from one to two days, objects whirled from left to right, he staggered and often fell toward the right Turning the head

either to the right or left did not initiate an attack, but always increased the vertigo when present. These attacks gradually became less severe, and after six months ceased entirely, but left him with a constant high pitched tinnitus and a slowly progressing deafness in the right ear. Then for a period of twelve years he had no vertigo. Eight weeks before admission to the hospital the tinnitus in the right ear became much louder, (a combination of roaring, buzzing and ringing), and the deafness increased. A fortnight later he began to have dizziness three or four times a day, and a feeling of fullness in the right ear. Then suddenly the violent attacks of vertigo, identical with those of twelve years earlier, began. Objects whirled to the right, in the horizontal plane, and he fell toward the right. The first severe attack came while sitting at his desk writing; he was thrown to the floor but did not lose consciousness.

He had had an operation for tuberculous epididymitis in 1920, and a tuberculous kidney on the right was removed in 1934. He never had an ear or sinus infection, no headache or allergic history. The general physical and neurological examinations were negative. Blood pressure was 110/80. Height 64½ inches; weight 135 pounds. The tympanic membranes and eustachian tubes were normal. The Weber test was referred to the left or good ear. Air conduction was better than bone conduction. Bone conduction time was normal on the left and nil on the right. The vestibular caloric test was normal on the left, and present but subnormal on the right.

His entire auditory nerve on the right was divided. When examined four years later he was perfectly well. The vertigo attacks and the dizziness with quick movements of the head had disappeared, but the tinnitus was unchanged and still referred to the right ear, although the nerve had been cut and he was totally deaf in that ear. The feeling of fullness in the right ear had also disappeared. The left ear was normal in every way.

Discussion. Severe Ménière attacks are rare in children, but we have seen them develop in another patient at the age of six, and continue with increasing severity until relieved by operation ten years later.

DEAFNESS AND TINNITUS

The deafness of Ménière's disease has all the characteristics of an inner ear or cochlear nerve lesion. In the absence of post-mortem examinations, we can only speculate about its cause and location. It could be in the hair cells of Corti's organ, in the non-medullated nerve

fibers that ramify among the cells of Corti's organ, in the ganglion cells of the cochlear nerve, in the trunk of the cochlear nerve, or in the cochlear nuclei. Very little is known about the effect on hearing of more centrally located lesions. A few years ago we made audiometer and tuning fork tests on several patients after removal, at operation, of the entire right temporal lobe or cerebral hemisphere for infiltrating glioma, and found the hearing normal in both ears (8). After removal of the left temporal lobe, aphasia makes such an examination impossible. It seems impossible that the deafness in Ménière's disease could be due to a lesion in the cerebrum, and equally impossible that a lesion in the mid brain or hind brain could so profoundly affect the auditory and vestibular tracts, without ever involving other structures.

Vertigo is the chief symptom in Ménière's disease, but deafness and tinnitus are so common that they must be regarded as part of the clinical picture. It is probable that all symptoms of this disease arise from the same source. There is nothing about the tinnitus of Ménière's disease to distinguish it from that seen in middle ear conditions, such as otosclerosis. Mygind (9) thinks that tinnitus is due to an intracellular edema of Corti's organ. If this were so the tinnitus as well as the vertigo should disappear, which it does not, after cutting the entire eighth nerve. The vertigo attacks always stop, but in 25 patients examined several years after this type of operation, the tinnitus was still present, unchanged or worse, in 17 or 68 per cent. These findings support the theory that the lesion is in the nerve and not in the peripheral end organs.

The diagnosis is based largely on the history of the attack. Tinnitus and impaired hearing are usually present, but their absence does not preclude a diagnosis of Ménière's disease. Of 93 patients cured of vertigo by operation, eight had no tinnitus at any time, but did have partial deafness on the affected side. Either intermittent or constant tinnitus in one ear may be the deciding factor in directing the operative therapy to that ear. This symptom is particularly helpful if the hearing is normal on both sides, as it was in five, or impaired on both sides as in thirteen of 146 patients.

The deafness is always of the inner ear type, is usually progressive, and has the peculiar characteristic of fluctuating. In this respect

it differs from inner ear deafness due to all other causes. A severe attack of vertigo usually precedes remissions of deafness and tinnitus;

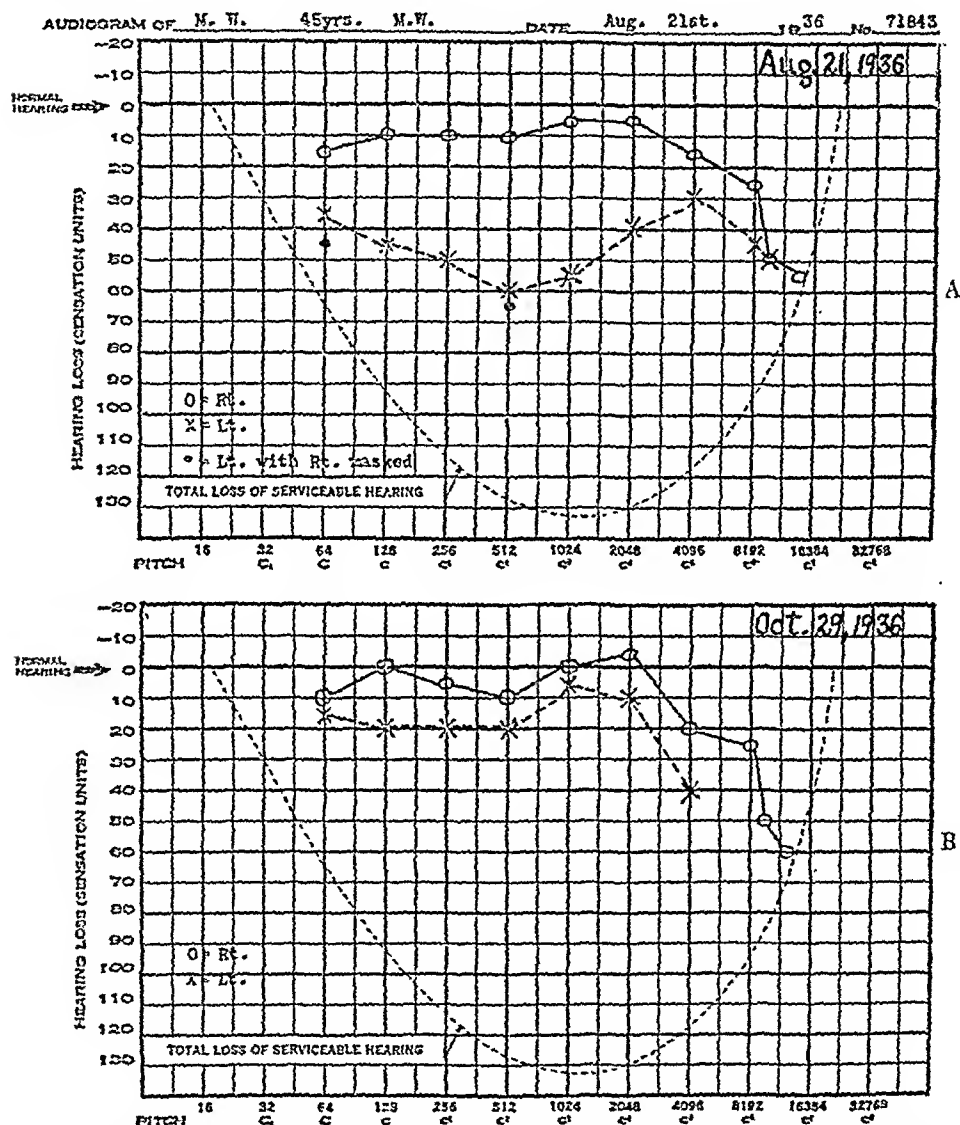


FIG. 6. Audiogram (A), made just before the intracranial division of the left vestibular nerve for Ménière's disease. (B) was made about three months later. Note the marked improvement. He now has normal hearing for conversation on both sides. Also note the loss of an octave and a half at the high end of the scale, due to the division of some cochlear fibres.

sometimes, however, a marked improvement in hearing follows an operation, at which nothing is done but to expose the eighth nerve,

and divide its vestibular fibers. Figures 6A and 6B shows improved hearing after operation. A remission in deafness is usually associated with a decrease or total disappearance of tinnitus, but on the other hand the deafness may remain unchanged, or continue to progress, after the tinnitus has stopped. These facts cannot be explained until we know more about the pathology of Ménière's disease, but they suggest changes in the end organs rather than the nerve or central pathways.

The following case history illustrates fluctuation in hearing, and several other interesting features of this disease!

G. B. K. (No. 60337). A white man, aged 50, entered the hospital in January, 1935, complaining of vertigo, tinnitus and recurring periods of deafness in the right ear. Since early childhood he had been totally deaf in the left ear. He has had no tinnitus at any time in the left or deaf ear, but for 30 years there have been recurring periods of tinnitus, "a hissing sound like escaping steam," in the right ear. Until eight months ago, however, these attacks were brief and never more frequent than three or four times a year. At that time for no known cause the symptoms changed. The tinnitus became constant and he began to have recurring periods of deafness. As he described it, "sounds are jumbled and indistinct like a radio out of tune." These periods sometimes lasted for several weeks with increasing deafness, and culminated in a sudden, severe attack of vertigo. He often fell and lost consciousness. At times objects seemed to whirl from left to right, and at other times in the vertical plane. After such an attack the hearing always cleared.

The vertigo is now more constant and severe, and it is impossible for him to work. There is no history of infection in the ears or upper air passages, and his eustachian tubes are normal. He has no headache, no digestive disorder, and his general health is good. The physical and neurological examination is negative. Blood pressure 128/85. Tympanic membranes look normal. Total deafness on the left. On the right, air conduction is better than bone conduction, and the bone conduction time is markedly shortened. The reaction to the vestibular caloric test indicates that the end organs in the semicircular canals, and the vestibular nerve on the left arc intact, although this ear is totally deaf. The response is subnormal on the right.

The entire eighth nerve on the left was cut. Although there was total deafness in this ear, the vestibular apparatus was still vital. When last

examined $2\frac{1}{3}$ years after operation, he still had tinnitus, and recurring periods of deafness in the right ear, but no vertigo. These symptoms must be due to Ménière's disease, without vertigo. The patient is well and works regularly.

Discussion. This patient had recurring periods of tinnitus for 30 years before the deafness and vertigo began. If this was an isolated case we would conclude that the history was a coincidence and that the tinnitus was due to some other cause. In reviewing the history of 117 of our cases of Ménière's disease, however, we find that the first symptom was:

	<i>Number of cases</i>
A sudden and characteristic attack of vertigo in.....	74
Deafness, usually unilateral and often with an abrupt onset.....	14
Tinnitus	31

Otologists and neurologists often see patients with an unexplained deafness of the inner ear type, or very annoying unilateral tinnitus without other symptoms. These symptoms develop so often into Ménière's disease that the primary cause is probably the same, the difference being due to the extent and location of the disease.

Illustrating onset of Ménière's disease with impaired hearing which has steadily progressed, and with fluctuating vestibular responses

W. M. (No. 59869). The first symptom of this man, aged 61 years, was a "feeling of fullness" and impaired hearing in his right ear. This was four months before admission to the hospital. There was no pain or infection in his ears at any time. Tinnitus in the right ear and vertigo came on simultaneously two months later and without warning or apparent cause. The tinnitus was a combination of a roar and a high pitched ringing and has been constant since its onset. The average duration of the vertigo attacks was three hours; objects whirled from left to right, there was no loss of consciousness, no headache, nausea or vomiting at any time. He has had five major and numerous minor attacks. The minor attacks consisted of a momentary feeling of unsteadiness. He says he felt and walked as if he were intoxicated.

The general physical examination was negative with the exception of a small adenoma of the thyroid and some peripheral and retinal arteriosclerosis. Blood pressure 196/80. Weight 130 pounds. Height 5 feet 8 inches. The neurological examination was negative with the exception

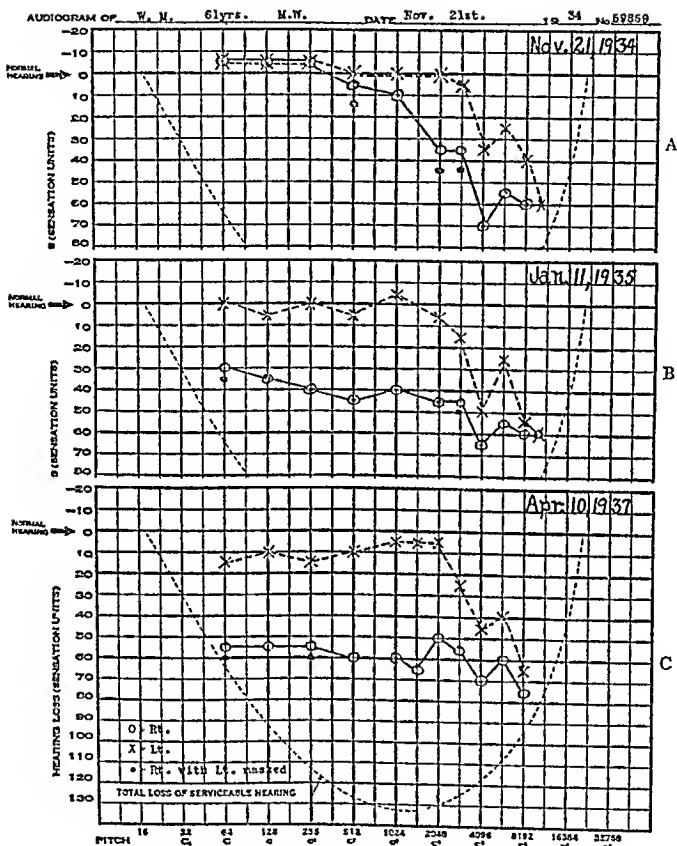


FIG. 7. These three audiograms show progressive loss of hearing on the right. When audiogram (A) was made the patient had noticed some hearing impairment on the right for four months. Note that the high tones are impaired and the low tones are normal. The deafness progressed rapidly as shown by audiograms (B) and (C). By progression we mean the hearing for low tones became more and more impaired. Hearing by bone conduction for the 512 fork was normal on the two sides in (A), and normal on the right, but nil on the left when the audiograms (B) and (C) were made.

of the eighth nerve on the right side. There was no infection in the upper air passages. The tympanic membranes were normal. The Weber test was referred to the left or good ear. Air conduction was better than bone conduction on both sides. The audiometer chart made in November, 1934, shows a bilateral impairment for the high tones, more marked on the right side (fig. 7). Intracranial division of the vestibular part of the auditory nerve on the right. The last examination was $2\frac{1}{4}$ years after the operation. He was working regularly and had had no vertigo or dizziness of any kind since the operation. "Rattling a newspaper sounds like breaking glass balls" in the right ear. No trouble of any kind with the left ear. "No staggering or uncertainty when walking, even in the dark." The deafness on the right is progressing (fig. 7C). The tinnitus is the same as before operation.

Discussion. This case illustrates the gradual onset of Ménière's disease with unilateral ear symptoms. The tinnitus appeared in association with the first attack of vertigo, and two months after the hearing impairment was noticed. The audiograms show that the high tones were first affected, and that during the past $2\frac{1}{4}$ years the low tones have become more and more impaired (fig. 7). Since division of the vestibular nerve on the right all vestibular symptoms have disappeared, but the auditory symptoms have become progressively worse.

Before operation the vestibular caloric tests were normal on both sides. After operation, no response was obtained on either side, even after 60 cc. of ice water were used. Another vestibular test was made two and a quarter years later, and the response on the left was present and very active, but incomplete. The nystagmus and falling reactions appeared promptly, but there was no pointing reaction with the right arm and no subjective sensation of dizziness. This and other cases, in which the vestibular response has been nil at one examination and present at a subsequent one, proves that vestibular functional tests are not always reliable.

Illustrating Ménière's disease with vertigo, but no deafness and no tinnitus

J. R. (H 248), a business man, 45 years of age, had vertigo for the first time about two weeks before examination. The onset was sudden with no warning; objects whirled toward the left, and he had nausea and staggering. There was no tinnitus and no impairment of hearing. No history of infec-

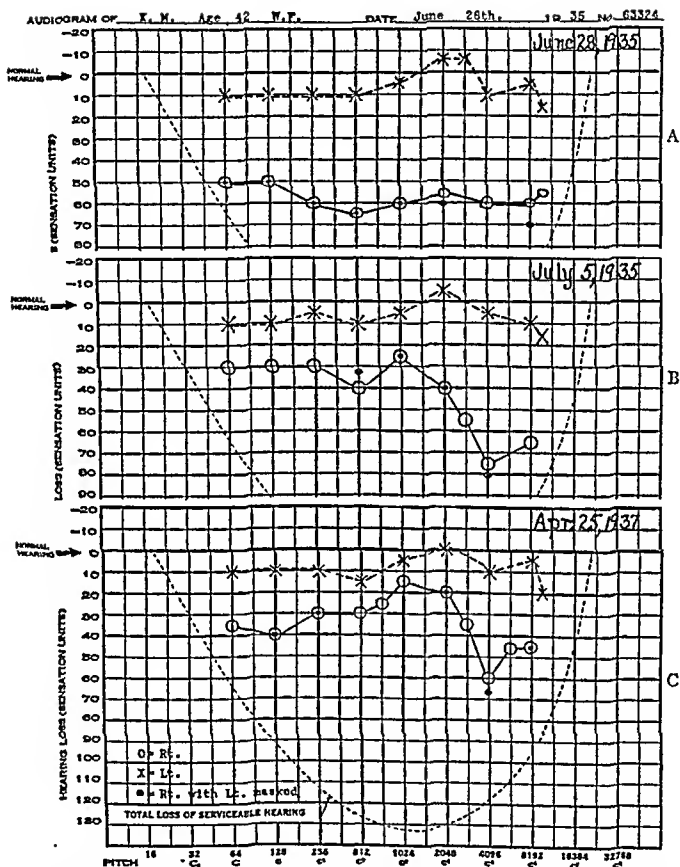


FIG. 8. These three audiograms show improvement in hearing following operation. (A) was made the day preceding the operation; (B) was made one week later, and (C) almost two years after the operation. In all tests air conduction for the 512 fork was better than bone conduction. The hearing by bone conduction was nil on the right at the time of the first examination (A), but had returned and was heard in this ear from 8 to 10 seconds one week after the operation (B).

tion or trouble of any kind with his ears. The vertigo attacks were sometimes momentary and sometimes severe, lasting for several hours.

His general physical examination was negative, but he had had no vacation for three years, had insomnia due to financial worries and the depression, had smoked to excess and drank five large cups of coffee daily.

The neurological examination was negative. Nothing abnormal was seen in the ears or upper air passages.

Air conduction was better than bone conduction and bone conduction was normal on both sides. The vestibular caloric test was normal on both sides.

Illustrating improvement in hearing after operation

K. M. (No. 63324), a trained nurse, aged 42 years, whose first symptom was deafness in her right ear three years before examination. Tinnitus also began at this time. Recently the deafness has progressed rapidly. The first attack of vertigo was a year after the onset of the deafness. There was then a period of about a year during which she had no tinnitus, no dizziness, and in which the hearing improved. The second attack came without warning, was very severe, and for three weeks before admission she had had almost constant dizziness. The general physical and neurological examinations were negative.

Operation, June, 1935. Section of the vestibular part of the eighth nerve on the right. An audiometer test made seven days after the operation showed marked improvement in hearing for 2048 d. v. and all lower tones (fig. 8). When last seen nearly two years later she was working regularly, was perfectly well, the tinnitus had disappeared, and the hearing in the right ear has continued to improve.

Discussion. The first symptoms were due to irritation and impaired function of the auditory apparatus, and only after a year did the irritant extend to the vestibular structures in a concentration sufficient to produce clinical symptoms. Following the first attack of vertigo, which was quite severe, there was a spontaneous remission of all auditory and vestibular symptoms for a year. Following the intracranial division of the vestibular nerve, all vestibular symptoms disappeared, as they always do, but in addition, the auditory symptoms again improved and the improvement has been maintained for two years. It is evident that something about the operation reproduced the conditions that brought about the spontaneous remission

a year previously. These facts preclude the possibility of a destructive lesion being the cause of the symptoms, but suggest on the other hand, a dysfunction of the endolymph.

SUMMARY

We believe that stimulation of the vestibular end organs is the cause of the sudden, violent attacks of vertigo, the sensation of surrounding objects whirling and the temporary staggering gait in Ménière's disease. When the central vestibular pathways are irritated by a new growth, the dizziness and incoördination are constant, there is no sensation of objects spinning in a rotary direction, and the periods of freedom from all vestibular symptoms so characteristic of Ménière's disease, are conspicuously absent. In other words we believe that Ménière's disease is a form of aural vertigo that involves the cochlea as well as the static labyrinth.

We are led to these conclusions for the following reasons:

1. The vestibular symptoms of Ménière's disease differ only in severity from those produced by functional tests. Otherwisc they are identical.

2. The infrequency with which these symptoms are seen in cases with acoustic, brain stem or cerebral tumors argues against the primary cause of Ménière's disease being in the nerve or central vestibular pathways.

3. Involvement of cranial nerves, other than the eighth, is rare: another indication that the lesion causing Ménière's disease is in the peripheral auditory and vestibular structures in the temporal bone, and not in the brain stem.

4. Choked disc or evidence of increased intracranial pressure is never present in Ménière's disease.

5. In five patients with acute Ménière's disease, the vestibular nerve was cut under local anesthesia. In two no sensation was noted when the nerve was divided, two complained of a momentary sensation of dizziness, and one heard a loud noise like thunder, but experienced no sensation of vertigo. In other words pinching or traumatizing the vestibular nerve in patients with severe Ménière's disease, fails to produce anything resembling an attack of vertigo.

6. In two patients the entire eighth nerve was cut and a piece of the

nerve removed for histological study. It was always found to be normal. Furstenberg, at the University of Michigan, has examined a piece of the auditory nerve removed at operation in eleven cases of Ménière's disease, and arrived at the same conclusion.

7. The auditory nerve between the labyrinth and brain stem is in contact with the facial and the nervous intermedius. These nerves are never involved in Ménière's disease. If the lesion were a neuritis, the adjacent nerves would occasionally be involved.

8. When the severe, characteristic attacks of vertigo recur after operation, they are due either to incomplete division of the vestibular nerve, or involvement of the opposite side, and a second operation stops the attacks.

9. Penfield (10) using a local anaesthetic, exposed the cerebral cortex and found local areas that when stimulated gave rise to a sensation of dizziness and falling. Similar results are reported by Foerster. So far as we know, this is the only evidence in favor of the cerebral origin of vertigo. No one has proved that deafness of the type so common in Ménière's disease can arise from a cerebral lesion.

10. Hardy and Crowe (11) reported six cases with small tumors and ten with angiomatous masses arising within the cochlear or vestibular nerve, many of them in the ganglion of the vestibular nerve. These patients had no vestibular or auditory symptoms of any kind, due we suppose to the fact that the growths were so small that there was no pressure on the nerve or interference with the blood supply. The minute tumors were missed at autopsy, and were discovered during a routine study of serial sections of 250 temporal bones. Employing the same method of study, metastatic growths in the eighth nerve have been found in other patients who had no symptoms referable to the ears.

11. Post-operative observations on Ménière's patients, in some cases for as long as nine years, have convinced us that after section of the vestibular nerve it is impossible to obtain a reaction with a caloric or turning test, due to the fact that the end organs in the semicircular canals are no longer connected with the brain. For the same reason, it is impossible for the patient to continue to have the vestibular

symptoms of Ménière's disease after a successful operation. If the primary lesion giving rise to the symptoms of this disease were in the central nervous system, or in the central end of the cut nerve, one would expect some symptoms to persist. The majority of these patients are now working and are perfectly well.

12. The strongest reason for our thinking that Ménière's disease is the result of pressure or chemical changes in the endolymph, is the frequency and length of the remissions. Anatomically the endolymph, and possibly the perilymph, are the only common factors in which a disorder, or one lesion, could affect both the organ of Corti and the vestibular end organs. With a remission, all of the cardinal symptoms of the disease may disappear for months or even years, and suddenly return for no apparent reason. As a group, these patients are extraordinarily free from general systemic disorders, such as cardio-renal disease, arteriosclerosis, focal infections or allergic disease. Of 117 cases in our group, only five had a history of allergy or migraine; and only two had a positive Wassermann test.

These facts lend interest to the observations of Mygind and Dederding (12), who think the symptoms of Ménière's disease are due to faulty water metabolism, and the experiences of Furstenberg, who thinks the sodium ion is the exciting cause. Furstenberg writes in July, 1937:³ "We have now treated approximately one hundred and twenty-five patients with such splendid results that we continue to maintain an interest and enthusiasm in our therapeutic researches. We have found it necessary, however, to hospitalize these patients for a period of a week or ten days in order that we may train them to use the proper diet, but more particularly to refrain from taking anything which contains sodium. So frequently we were disappointed when treating patients out of the hospital because they continued to take sodium bromide, "for their nerves," or sodium bicarbonate "for gas on the stomach." We discovered, also, that the source of the patient's water supply was exceedingly important. When several of our patients failed to respond to the treatment, we discovered that they were drinking water from deep artesian wells which contained about thirty grains of sodium chloride to the quart. Our only dis-

³ Personal communication

couraging results have been among those individuals whom we were unable to keep under close observation in the hospital.

"In a large proportion of our patients the tinnitus was relieved but I don't believe any of them experienced a cure. With a reduction in the intensity of the tinnitus, the hearing for conversational voice likewise improved in some of our patients. It is doubtful, however, that the treatment has any effect upon the hearing although it seems to reduce the tinnitus in a rather large proportion of cases."

In this connection the observations of Loeb, Harrop and others are of interest. They find that the cortex of the adrenal glands controls sodium metabolism in much the same way as calcium is controlled by the parathyroids.

If the symptoms of Ménière's disease were due to a disorder in the metabolism of sodium or fluids, why should patients get perfectly well after division of one vestibular nerve with no change in their dietary habits?

Why should the disease involve only one ear in the majority of cases? How are the spontaneous remissions explained? Also how can the variations in hearing be explained? In some patients tinnitus is the only symptom for months or even years preceding the vertigo; others have no tinnitus, no deafness but repeated and violent attacks of vertigo. Finally it seems impossible to explain on this theory the following facts observed by us at the post-operative examination of 72 patients

1. The deafness in the affected ear was worse in 22 (30.5 per cent) of 72 cases.
2. The deafness in the affected ear was unchanged in 36 (50 per cent) of 72 cases.
3. The deafness in the affected ear was much improved in 14 (22.2 per cent) of 72 cases.

The average elapsed time between the operation and the last examination was 2.2 years.

Ménière's disease is a common malady. It is a combination of vestibular and auditory disorders. The diagnosis is not difficult, and is made largely on the description of the vertigo attacks. Too much emphasis placed on special examinations, such as the audiometer,

tuning fork and vestibular tests may lead to confusion. The diagnosis of Ménière's disease does not depend on the demonstration of impaired hearing, shortened bone conduction, or an abnormal vestibular response to caloric or turning tests. This disease may occur in patients with infections, brain tumor, arteriosclerosis, syphilis, or other systemic diseases, but so infrequently that we feel sure none of these conditions is the primary cause. It is a disease of middle life; of 138 patients, 110 were between the ages of 30 and 60 years, when the first symptom appeared. The average blood pressure of 68 operated cases was 137/83. The average weight of 47 operated patients was 155 pounds. Of 117 patients 74 were male, and 43 female; 115 were white and 2 colored. Only 10 of 71 patients had a history of otitis media at any time, and only 2 of 121 had an infection of the accessory nasal sinuses, when admitted to the hospital.

Vertigo is the symptom that disables these patients. It is always cured by an intracranial division of the vestibular nerve. The deafness and tinnitus are only slightly less disturbing. Histologic demonstration of the pathology of Ménière's disease, and a clear understanding of its cause, will also explain many other obscure affections of the inner ear, such as tinnitus and progressive inner ear deafness without vertigo.

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wherever used internally is in large measure referable to its radioactive properties, an understanding of the radioactivity of thorotrast is essential in any discussion concerning the advisability of its use clinically.

RADIOACTIVITY

As pointed out by Flinn (103), thorotrast has been advocated for roentgenological purposes by persons not familiar with the action of radioactive material.

Thorotrast is a stabilized emulsion of thorium dioxide containing 25 per cent by volume, and 19 to 20 per cent by weight of thorium dioxide, and about the same amount of protective colloid, from 16 to 19 per cent by weight. This colloid is of a carbohydrate nature and defined further as a dextrin preparation. As a preservative it contains 0.15 per cent of methyl-p-hydroxy-benzoate (96).

Thorotrast can be shown to be radioactive by means of the electroscope. This state of radioactivity is not altered by injection into the body, for it is easily demonstrated by electroscopic tests on animals which have been injected with this material several months previously (103).

The radioactivity of thorium closely resembles that of radium in that this substance breaks down into the subproducts of mesothorium, radiothorium, thorium X, thorium emanation, thorium A, B, and C, which, as they are formed, in turn give off alpha, beta, and gamma rays. A radioactive substance then is constantly undergoing spontaneous disintegration in the formation of a new atom distinct in physical and chemical properties from its parent substances (98).

If the radioactivity of freshly isolated thorium is taken as 100 per cent (considering only the emission of alpha particles) in from four to five years, it will decrease to about 50 per cent of the initial level. This is due to the fact that radio-thorium, one of the disintegration products of mesothorium, is chemically inseparable from thorium.

During the time radiothorium is disintegrating, the concentration of mesothorium (which does not emit Alpha particles) is constantly increasing. At the end of the four or five year period, enough radiothorium is regenerated again from the mesothorium to increase the emission of alpha particles.

TABLE 1
Radium disintegration series

ELEMENT	ATOMIC WEIGHT	ATOMIC NUMBER	TYPE OF DISINTEGRATION	TIME (HALF PERIOD)
Uranium 1	238.18	92	Alpha	4.5×10^9 years
Uranium X ₁	(234)	90	Beta-gamma	24.5 days
Uranium X ₂	(234)	91	Beta-gamma	1.14 minutes
Uranium Z	(234)	91	Beta	6.7 hours
Uranium 11	(234)	92	Alpha	2×10^6 years
Uranium Y	(?)	90	Beta	24.6 hours
Ionium	(230)	90	Alpha	7.6×10^4 years
Radium	225.97	88	Alpha-beta	1,600 years
Radon	(222)	86	Alpha	3,825 days
Radium A	(218)	84	Alpha	3.05 minutes
Radium B	(214)	82	Beta-gamma	26.8 minutes
Radium C	(214)	83	Alpha-beta	
			Gamma	19.7 minutes
Radium C'	(214)	84	Alpha	10^{-6} seconds
Radium C''	(210)	81	Beta	1.32 minutes
Radium B	(210)	82	Beta-gamma	25 years
Radium E	(210)	83	Beta-gamma	5 days
Radium F (Polonium)	(210)	84	Alpha	133.3 days
Radium G (radium lead)	(206.05)	82	Stable	

Thorium disintegration series

Thorium	232.12	90	Alpha	1.65×10^{10} years
Mesothorium 1	(228)	88	Beta	6.7 years
Mesothorium 2	(228)	89	Beta-gamma	6.13 hours
Radiothorium	(228)	90	Alpha-beta	1.9 years
Thorium X	(224)	88	Alpha	3.64 days
Thoron	(220)	86	Alpha	54.5 seconds
Thorium A	(216)	84	Alpha	0.145 seconds
Thorium B	(212)	82	Beta-gamma	10.6 hours
Thorium C	(212)	83	Alpha beta	60.5 minutes
Thorium C'	(212)	84	Alpha	10^{-11} seconds
Thorium C''	(208)	81	Beta-gamma	3.20 minutes
Thorium B (thorium lead)	(207.77)	82	Stable	

Compiled from Fajans 1922, and Rutherford 1930.

In seven years about one third of the difference is made up, in ten years about one half. In order to have thorotrast at its minimal level of alpha radioactivity, it would have to be made from thorium aged

four or five years. Deleterious effects from the delayed radioactivity might not become apparent for four or five years after the administration of the material and might conceivably be delayed as long as nine or ten years (98).

The relation of thorium to its disintegration products is shown in table 1. The similarity of the Thorium Series with that of the Radium Disintegration Series is quite apparent.

In view of the fact that the alpha ray is the one primarily responsible for damage when radioactive materials are stored within the body, a consideration of some of the characteristics of these particles is relevant at this time.

THE ALPHA PARTICLES

Of the total energy emitted by radioactive substances, about 95 per cent is carried by the alpha particle. In the usual application of radioactive materials this ray or particle is unimportant because it is filtered out easily and its range is short. Regardless of their source, even a millimeter of tissue will absorb most of these rays. The storage of radioactive materials internally, such as occurs after an intravenous or cerebrospinal injection of thorotrast, provides an opportunity for the damaging effect of the alpha radiation not previously experienced in the usual external methods of applying radioactivity.

The relatively tremendous destructive power of the alpha particles was first demonstrated in an early paper by Willcock (97). Attention clinically, however, was really first aroused by the occurrence of the cases of occupational poisoning associated with the manufacture of luminous watch dials (101, 115).

The alpha particles consist of doubly positively charged helium atoms ejected from radioactive bodies with a velocity of $1/12$ th to $1/20$ th that of light, or approximately 18,000 to 12,000 miles per second. Although their velocity depends upon the body from which they are expelled, from any one particular substance they are ejected with the same velocity.

Biologically, the alpha rays are more destructive than either the beta or gamma rays. The relative ionization caused by the alpha, beta and gamma rays will be found to be approximately 10,000, 100, and 1, whereas the relative penetrations of these rays is in the reverse

order, the beta rays going about 100 times as far as the alpha rays, and some of the gamma rays about 100 times as far as the beta rays. For this reason, radioactive elements in such small quantities that the beta and gamma radiations are almost negligible still produce, by means of their alpha radiations, intense physiological effects if given by mouth, vein or cerebrospinally (97, 111, 114).

The important consideration is that the ionization due to alpha radiation is locally an intense one. That due to the beta rays is less intense and less localized, while gamma rays have a small ionizing action, which may, however, be distributed over a very large space.

Of importance, too, is the fact that mesothorium in equilibrium with its radiothorium emits five alpha particles, whereas radium only emits four. Moreover, the alpha particles of mesothorium and the products of its decay have a greater velocity and penetrating power than those of radium, and therefore are chemophysically and physiologically more active (115).

PATHOLOGICAL EFFECTS OF THORIUM AND THE ALPHA RAYS

The relatively tremendous destructive power of the alpha particles was first demonstrated in an early paper by Willcock who exposed *Hydra viridis* to 50 mgm. of radium bromide in such a way that alpha, beta and gamma rays could all reach the organism. The vitality of the animal was low at the end of one hour and disintegration occurred after two hours. When only beta and gamma rays from the same preparation were allowed to reach the organism, the latter survived for several days after an exposure of four and a half hours (97).

Attention clinically, however, was really first aroused by the occurrence of the cases of occupational poisoning associated with the manufacture of luminous watch dials (101, 115).

From 1922 to 1928 thirteen deaths occurred which were designated by Martland as early cases, the outstanding clinical feature of which was an intense radiation osteitis, particularly of the jaw, and an anemia of the regenerative type. The late cases escaped the extensive necrosis of the jaw and the fatal leucopenic anemias, developing instead chronic, crippling bone lesions, which occurred often after several years of good health, and affected primarily the bones subject to trauma. The anemias were of a milder variety (114).

The paint used in the cases consisted of crystalline phosphorescent zinc sulphide rendered luminous by the addition of small amounts of radium mesothorium and radiothorium. The majority of the workers affected had swallowed the paint for periods of one to four years or more by pointing their brushes repeatedly in their mouths (114).

Electroscopic studies showed the mesothorium predominated in the early cases, while only radium was detected in the post-mortem examinations of the late cases. The preponderance of mesothorium in the luminous paint was felt to be of toxicological importance for the reason that mesothorium in equilibrium with its radiothorium emits five alpha particles, whereas radium only emits four. Moreover, the alpha particles of mesothorium and the products of its decay have a greater velocity and penetrating power than those of radium, and therefore are chemophysically and physiologically more active (114).

In the late cases a sufficient period after exposure, from six to seven years, had elapsed to allow mesothorium responsible for 70 per cent of the radioactivity to decrease in quantity by its own uninfluenceable decay to less than one half of its strength. These patients therefore seemed to be escaping the extensive necrosis of the jaw and the fatal leucopenic anemias (114).

Various investigators have reported the development of cases of aplastic anemias after the intravenous injection of relatively large amounts of thorium X, among them Laignel-Lavastine, P. George, E. Weil and Lacassagne (109, 121).

Martland called attention to the frequency of osteogenic sarcoma among the deaths of the dial painters. He attributed this to the chronic irritation from the radiation osteitis produced by the internal bombardment of the radioactive substances deposited in the bone (114).

Experimentally Sabin, Doan, and Forkner found that intravenous radium chloride and mesothorium were productive of osteogenic sarcoma in two out of seven rabbits surviving eleven to nineteen months (81).

Roussy, Oberling and Guerin produced peritoneal and subcutaneous sarcomas in eight of fifteen surviving mice that had received intraperitoneal and subcutaneous injections of 0.5 cc. of thorium dioxide

with a similar amount of saline every three to four days for a total of five injections (80).

Other experimental work, notably that of Fernau, Schramek, and Jarzicki, Lacassagne, Lattes and Levedan and De Silva, has shown the alpha rays to be productive of severe leucopenias associated with hemorrhagic purpuras, and acute nephritis (108, 116).

Andersen and Fischer, among others, invariably found that the alpha particles produced an inhibitory effect upon the growth of tissue culture (93, 94).

Zirkle (121, 123), Holweck, and Lacassagne (105, 106) reported a similar effect upon the germination of various spores, and Chambers and Russ (97) discovered that the alpha particles hemolyzed red corpuscles.

With injections of 1 m.c. or more of a radium chloride solution through the cornea, Altschul has demonstrated various degrees of inflammatory changes in the eye (92).

As a result of thorium poisoning Flinn has reported a case of cataract development with complete blindness, an eye condition which also developed in some of his experimental animals (101).

The time for a general theory to explain the mode of action of radiation upon living tissue has hardly arrived. In early years, nuclear damage was favored. On other occasions, some particular cell constituent, for example, lecithin was thought to be decomposed by radiations, and the changes attributed either wholly or in part to the action of its decomposition products.

One explanation of a mode of action of the radiation upon living tissues is that the effect is one which disturbs the normal equilibrium of the irradiated parts. Generally speaking, small doses of radiation give rise to transient effects, while more intense radiation produces permanent damage or complete destruction (95).

RADIOACTIVITY OF THOROTRAST

The alpha ray activity of 25 cc. of thorotrast has been found to be equivalent to a maximum of 1 microgram and a minimum of 0.5 microgram of radium. The beta and gamma rays are too feeble to be of any physiological significance. Should no elimination take place,

one would obtain an accumulated alpha ray activity of from 1.5 to 5 micrograms of radium with a maximum dose of 75 cc. of thorotrast (96).

Although the limit of tolerance for the average person was formerly thought to be about 10 micrograms of radium, bone and teeth changes have been observed in the bodies of radium workers which contained the equivalent of from 2 to 4 micrograms of radium. Moreover, Leake found roentgenographic evidence of focal atrophy and sclerosis of the mandible in some radium dial workers possessing 1 microgram of radium. Therein lies the potential element of danger in the use of thorotrast.

HEPATOLIENOGRAPHY

Following the first use of a colloidal preparation of thorium dioxide for bronchoradiography in 1928 by Blühbaum, Frik, and Kalkbrenner (46) Oka (6) in 1929, accidentally discovered the outlining of the liver and spleen roentgenologically following the intravenous injection of the colloidal thorium dioxide substance, called "Tordiol." In the same year, this work was independently verified by Radt (67) who improved the preparation, used it clinically and introduced it as "thorotrast."

In due time various investigators reported rather enthusiastically their excellent radiographic demonstrations of the spleen and liver obtained with intravenous injections of thorotrast. Among them were those of Radt (66), Bauke (44), Popper and Klein (64), Naegeli (76), Einhorn, Stewart, Illik, and Kadrnka (50). Along with their expressions of enthusiasm, however, there appeared not infrequently a more critical attitude concerning the advisability of using a heavy radioactive metal intravenously, an attitude which became more pronounced later on.

REACTIONS

Variable reactions from the intravenous injection of thorotrast have been reported. These have included slight to moderate febrile manifestations, vomiting in hepatic cirrhosis, sometimes lasting several days, anaphylactic phenomena with particularly severe asthmatic attacks in individuals so disposed, and a few cases resulting in purpuric manifestations (96).

ELIMINATION

The elimination of thorotrast is insignificant. The thorium dioxide remains more or less indefinitely in the cells of the reticulo-endothelial system, particularly in the spleen and liver. According to the work of Leipert, the spleen and liver take up two-thirds of the injected material, and of this the spleen stores nine times as much of the thorium dioxide as the liver. Thereafter, the thorotrast was found regularly in the red marrow, and in the lungs, so that the sequence of storage is spleen, liver, marrow and lungs (60).

Although Kadrnka (60) and Irwin (56) have postulated an elimination of thorotrast through the lungs by means of the bronchial secretions, Leipert (60) has emphasized that even if one were to consider the accumulation of thorotrast in the lungs significant, one would have to admit that the stored quantities in the spleen and liver had remained uninfluenced. At all events, any elimination by means of bronchial secretion is productive of no significant decline of thorium dioxide content of the remaining organs.

Fecal and urinary examinations after the injections of thorotrast, as reported by Leipert, have given negative results both chemically and electroscopically in animals followed more than one and a half months (60).

Over periods of months most investigators have found no significant change in the radiopacity of the organs, among them Angermann and Overhof (42), Blühbaum, Frik and Kalkbrenner (46), Huguenin, Nemours and Albat (55), Pohle and Ritchie (63), Popper and Klein (64), Einhorn, Stewart and Illick (50) and Tripoli (69). After observations of three years, Naegeli and Lauche were unable to find any significant elimination of thorotrast from the body (76).

PATHOLOGICAL CHANGES

Although one seldom finds a unanimity of opinion regarding the pathological effects of thorotrast upon the reticulo-endothelial system, and particularly upon the spleen and liver, there can be little doubt that changes of a pathological nature occur. These changes vary with the amount of thorotrast injected and depend upon early as well as upon late effects.

Although not definitely proven, the early effects might well be due to the toxicity of the metallic salts themselves, or to a purely mechanical effect, and the late effects to their radioactivity, but at all events, both the mechanical and physical properties of thorotrast are concerned in the tissue responses.

Stages between an early hepatitis and the beginning of an experimental cirrhosis have been reported by Huguenin, Nemours and Albat (55). Variations from acute splenic tumor to splenic rupture with necrotic changes have also been described by Bungeler and Krautwig (48).

Fleming and Chase (100) have found the proliferative reaction to thorotrast greatest in localities where connective tissue is normally most abundant. Thus the fibrous tissue increase is much greater in periportal districts containing the metallic substance than about the central veins, although the substance is about equally distributed in both these regions. It was their impression that there was a considerable variation in the reaction or tolerance of different kinds of tissue and cells to this irritant.

Other investigators have been unable to find any significant pathological changes in the spleen and liver after thorotrast injection, but reports of pathological alteration have occurred much too frequently to be disregarded. Among them have been the publications of Anders and Leitner (41), Arrigoni and Porta (43), Baumann and Schilling (45), Bungeler and Krautwig (48), Fleming and Chase (100), Huguenin, Nemours and Albat (55), Kadrnka (57), Lambin (59), Pohle and Ritchie (63), Shute and Davis (68) and Tripoli and Haam (70).

FUNCTIONAL ALTERATIONS

Whether or not one speaks of a real or functional blockage of the reticulo-endothelial system remains essentially a quarrel of words. That there is a functional alteration seems undeniable.

After injections of thorotrast totalling 0.6 gram per kilogram of body weight, Tsunoo and Nakamura (71) found an absence of bilirubin formation for a period of two or three weeks.

By means of the Millon reaction and a modified functional galactose test, Popper and Scholl (65) and Huguenin, Nemours and Albat (55) reported an impairment of hepatic function.

Definite arresting of phagocytosis in organisms storing thorotrast has been observed by Hanke (53) and by Bucky and Leitner (47). In experimental animals infected with *Bacillus enteritidis* Held (54) noted a definitely retarding influence upon recovery when thorotrast was injected.

VALUE DIAGNOSTICALLY

As pointed out by Ebhardt (49), regardless of any question of a damaging effect of thorotrast, a negative result from hepatolienography does not with certainty eliminate the existence of small metastases. Conversely, the positively appearing X-rays are often subject to numerous sources of error. For such reasons, in any doubtful cases, and particularly in those justifying operative consideration, reliance, he felt, must still be placed upon exploratory laparotomy.

We believe therefore that if, after due consideration of the various clinical and experimental results of thorotrast hepatosplenography, one weighs the necessity and diagnostic value of the procedure against the probabilities of damage, both immediate and remote, one can hardly become enthusiastic about its application clinically.

EFFECTS UPON THE HEMOPOIETIC SYSTEM

Reports of the effect of intravenous thorotrast upon the blood picture have been numerous as well as varied. Although mechanical as well as physical properties of thorotrast are undoubtedly concerned in the reaction of tissues to this substance, and strictly speaking the two cannot be entirely separated, alterations more probably representative of its mechanical rather than its physical properties already discussed under radioactivity, will be considered at this time.

With doses of 0.5 cc. of thorotrast per kilogram, the usual amount in man, Lambin and Gerard (59) noted the occurrence of an initial shortlived thrombocytopenia. With larger doses the thrombocytopenia disappeared less rapidly. With doses of 2 cc. per kilogram, there occurred a tendency towards prolongation of the bleeding time during the first hour, while with 5 cc. per kilogram doses, prolongations of importance were observed.

Reinjections performed when the thrombocytopenia caused by the first injection had disappeared entirely, were productive of a similar result, though platelet regeneration sometimes occurred more rapidly.

Reinjections with a slight degree of thrombocytopenia still existing were apt to result in a lower count of blood platelets and a more prolonged bleeding time during the first day.

This indicates that thorotrast can produce more serious alterations in a thrombocytopenic organism than in the normal, and explains perhaps the more frequent occurrence of hemorrhagic accidents after thorotrast injections in those patients with a tendency towards bleeding.

In general it may be said that the white cells show an initial rapid leucopenic stage of short duration with a relative lymphocytosis, which is followed by a longer phase of leucocytosis accompanied by polynucleosis, after which there is a monocytosis.

Depending upon the size of the dose, there is a definite anemia, erythroblastic in type without reticulocytosis. An aplastic anemia has been produced in rabbits with an injection of 40 cc. of thorium dioxide per kilogram of body weight.

If thorotrast is injected five hours after the formation of antibodies, there occurs a diminution of hemolysins. Moreover, after the injection of thorotrast, a definite arresting of phagocytosis occurs. The phagocytic activity of the cells of the peritoneal exudate was found by Bucky and Leitner (47) to be reduced from 38 to 4 per cent.

These variations in the blood picture have been found to be quite analogous to those changes occurring after the injection of most colloidal substances and in no way can they be said to be peculiar to thorium dioxide.

THE LYMPHATICS

Thorotrast has been employed experimentally in the röntgenological demonstration of various lymphatic channels.

Menville and Ane (75) found changes in the lymph nodes not unlike normal cellular destruction and so extensive that they assumed their normal function had been destroyed. For this reason they speculated enthusiastically upon the use of thorotrast in the metastatic involvement of lymph nodes, and felt that it might produce an effect equivalent to a surgical removal.

Naegeli and Lauche (76) observed that strong concentrations of thorotrast were productive of enlarged necrotic areas in the lymph

nodes. They found that the granules of thorotrast became partially encapsulated and partially transferred to more distant nodes.

In the presence of an infectious or inflammatory process, Dotti (72) found that the shadows of the lymph nodes which developed after the subcutaneous injection of thorotrast were less even and intense and developed less rapidly than those which appeared in the normal animals.

In histological examinations following the intraperitoneal and intrapleural injections of thorotrast, Ruggieri and Zanetti (77) found that most of the organs examined showed vascular congestion, that the kidneys exhibited severe lesions of the glomerular tubules, the suprarenals zones of atrophy, the lungs numerous thromboses and the hepatic cells cirrhotic changes.

Harris (73) observed a distinct stimulating reaction upon the connective tissue or stromal unit of the node with loss of lymphoid structure. He also described a proliferation of the perivascular connective tissue, which continued to increase concentrically about the blood vessels. A more advanced picture revealed a complete loss of parenchyma with connective tissue replacement in which the lymph spaces were shown clearly and at times appeared dilated.

OSTEOMYELOGRAPHY

As pointed out by Sedgenidse and Solotuchin (83), the work of Anders, Askanay, Leitner, Blass, Kadrnka and Junet (82), Pohle and Corton Ritchie has shown that in most opaque demonstrations of the bone marrow with intravenous thorotrast, fractional doses totalling at least 5 cc. per kilogram were required, which in a 50 to 60 kilogram individual represents the excessive dose of 250 to 300 cc. of thorotrast.

On the other hand small aseptic bone removals not demonstrable by X-rays were visualized by Sedgenidse and Solotuchin (83) by means of the intravenous injection of only 2 or 3 cc. of thorotrast. In this way they felt that any pathological inflammatory or destructive bone lesion could be outlined.

PNEUMOALVEOLOGY

Kadrnka and Junet (82), using thorotrast intravenously in fractional doses totalling 15 cc. per kilogram of body weight, were able

to demonstrate the lung alveoli. They observed, moreover, the lung fields as a fine net, showing similarly large and regularly arranged granulations. With massive doses emboli were observed. After three or four months, the thorotrast had a tendency to be grouped in the bronchi, and more particularly in the peribronchial nodes. The large amounts of thorotrast required for the procedure, have, of course, prevented any clinical attempts in this direction.

The use of thorotrast in tuberculosis. The experimental work of Bennett revealed the inadvisability of using thorium dioxide in cases of tuberculosis because of the rapid dissemination of the disease through the tissues following the injection of this chemical (84).

PLACENTOGRAPHY

The importance of the normal and pathological location of the placenta, evident in ectopic pregnancy and placenta previa, and moreover the diagnostic problems of premature separation, hydatidiform mole and chorioepithelioma afforded important possibilities for the clinical application of thorotrast in placentography.

As far as can be determined, Ehrhardt in 1932 was the first to use thorotrast experimentally in placentography. During the course of his investigations, he was able to show that previously stored thorotrast had no influence upon conception, the course of pregnancy, nor upon the embryonic development. Rabbits, mice, rats, and guinea pigs, which had stored the thorium dioxide carried out the pregnancy and brought normal young into the world that later developed normally.

Injections performed during pregnancy so that thorotrast was stored in the placenta as well as in the spleen and liver produced somewhat different results.

In the experimental animals receiving small quantities of intravenous thorotrast during the first half of pregnancy, intrauterine death invariably occurred. During the second half of pregnancy some of the animals aborted while others carried on the pregnancy without disturbance, and the later development of their young occurred normally.

After the injection of thorotrast Ehrhardt observed that the placenta became demonstrable by X-ray before the spleen and liver were seen.

Even though the mother was the recipient of large quantities of thorium dioxide, he was not able to show the transportation of thorotrast from the placenta to the fetus by X-ray. Similarly, by means of appropriate injections, he was unable to demonstrate the transportation of thorotrast from fetus to mother.

A few hours after injection placental shadows of thorotrast were recognizable in complete intensity. During the following hours and days the intensity decreased, and in many cases almost completely disappeared. In other words, the thorium dioxide content of the placenta gradually became so diminished that the demonstration of the placenta by means of the X-ray was often no longer possible. Because of the placental remnants of thorotrast, however, the removed and isolated placenta still gave a more or less clear shadow (86, 87, 88).

Because of the frequency of abortion, and for the reason that the contraindications far outweigh any beneficial diagnostic possibilities, the clinical application of thorotrast for placentography has not met with any enthusiasm.

UROLOGY

For numerous reasons thorotrast seemed almost ideally suited for urological work, and its introduction was received with considerable enthusiasm. A most striking advantage has been the almost complete absence of pain associated with thorotrast pyelography. Because in the same concentration it absorbs more roentgen rays than sodium and lithium iodide, it can be used in a more dilute solution. It mixes readily with the urine contained in the organs, and thus allows their complete visibility. This substance was also found to be indifferent and painless for the mucous membranes. Moreover, should it enter the blood stream, it was felt that it would be relatively non-poisonous. It was also assumed that thorotrast would not flocculate, and could not therefore lead to obstruction. Furthermore, because of its lack of reaction, it was believed that the substance might be retained indefinitely in cystic or damaged kidneys without danger. Such, however, was soon found not to be the case.

Before long various investigators were writing about several disadvantages of thorotrast in pyelography, and within due time many felt, with reluctance, that this substance should be discarded in the field of urology.

Thorotrast is frequently retained more or less permanently in the kidney pelvis, and often, by means of reflux pyelovenous transportation becomes stored in the kidney parenchyma. Diagnostic errors from a confusion of the thorotrastic shadows with pathological kidney lesions occur, and too frequently have resulted in unnecessary nephrectomies. Moreover, parenchymal damage has been demonstrated histologically in the vicinity of the thorium dioxide deposits and serious obstruction to elimination has been noted in pyelography with hydronephrotic kidneys. Such disadvantages of the substance have been emphasized by Hennig and Lechnir (125, 126, 127), Krauss (129), Löhr and Hendriock (130), Puhl (131) Scheuer (134) and Viethen (135) and Scheele (132, 133). When the ever present danger of radioactivity is added to these findings the condemnation of its application for pyelography is all the more pronounced.

After pyelography with thorotrast, various investigators have noted a flaring up of a severe pyelonephritis from a latent kidney infection, associated with a complete ureteral blockage by the precipitated thorotrast.

By means of experiments in reagent glasses, and also upon living organisms, Hennig and Lechnir (126) have shown that in time thorotrast flocculates and precipitates when mixed with urine.

In spite of the almost ideal absence of pain associated with thorotrast pyelography and notwithstanding the initial enthusiastic reception of the material, the general opinion of the investigators concerned is that such pyelography with thorotrast is definitely contra-indicated.

THE CENTRAL NERVOUS SYSTEM

Cerebral angiography. The introduction of cerebral angiography with 25 per cent sodium iodide in June 1927 by Egas Moniz eventually resulted in such complications as palsies, disturbances of speech, not infrequent epileptic seizures and occasional fatalities. Moniz turned to thorotrast when this substance was being applied radiographically. It was brought to his attention in Lisbon, where it had been used with success in arteriography of the extremities. Moniz found that it gave much more satisfactory results and practically without the previous complications.

The method was improved by Pinto and Lima (22) and by Löhr

and Jacobi (19). These last named investigators combined ventriculography with arteriography in 1932.

According to the observations of Friedemann and Elkeles, and Jorns (15), after arterial injections of thorotrast, the thorium dioxide is not stored cerebrally even when small focal areas are made instrumentally. Instead, the substance is collected for the most part by the reticulo-endothelial cells of the spleen and liver.

From the standpoint of the arteriographic use of thorotrast in the diagnosis of tumors, it is difficult to believe that many cerebral neoplasms can ever been seen by an arterial change even if perfect injections were possible. Moreover, cerebellar tumors and vascular abnormalities are outside the limits of the carotid circulation, and would appear to be precluded from diagnosis unless the vertebral arteries were also injected. The occasions when arterial encephalography can justifiably be used in preference to or in conjunction with ventriculography for the diagnosis of tumors would seem to be decidedly limited.

At the present time thorotrast unfortunately is the most suitable means available for outlining vascular abnormalities. Untoward results have been infrequently reported by those familiar with the method. The procedure, however, particularly for those not accustomed to its use, possesses technical difficulties, and is one not exempt from danger.

If, after due consideration of the potentially damaging effects of thorotrast, there is any justification for its arterial injection, this would seem to be reserved for the diagnosis of vascular abnormalities in properly selected cases.

Thorotrast for encephalography and ventriculography. Krause was the first to attempt to put an opaque substance into the ventricles and spaces of the brain. The animals died at the conclusion of the injections. Independently of Krause, and in the same year, A. Simons filled the lumbar region of dogs with 0.5 to 20 per cent collargol solution. Inasmuch as the differentiation of the opaque substance was insufficient, outlining of the lumbar sac by X-ray was not successful. The experiments of Lippmann a year later were likewise unsatisfactory as were those of Berberich and Kirsch (39).

Dandy also used different concentrations of opaque substances in

the fluid spaces of dogs, including thorium, potassium iodide, collargol, argyrol, bismuth subnitrate and subcarbonate, but because of injurious effects upon the brain, his results were uniformly fatal. It was his opinion that no radiographic solution of value would be found which would be sufficiently harmless to justify its injection into the central nervous system, and that suspensions were precluded because of their non-absorbability (39, 5). Schuck expressed a similar point of view (39).

After the rather general application of thorotrast in various roentgenological procedures, it is scarcely surprising that intracranial experiments with this substance were undertaken.

Radvoci and Meller of France (23) and Wustmann of Germany (39) in March 1932 were the first to report upon the intracranial application of thorotrast.

Wustmann showed that the toxic effect of the first preparations of thorotrast, "1073 A", was due to a progressive elevation of the acidity of the cerebrospinal fluid following their injection, which resulted in convulsions and death. By means of various experiments, he was instrumental in developing thorotrast "1073 D", a more satisfactorily buffered preparation, which was well tolerated by his animals.

He pointed out that the results he obtained histologically were similar to those produced by others with the injection of various dye-stuffs. The thorotrast filled the subarachnoid spaces, outlining the contours of the cerebral and cerebellar hemispheres distinctly, and followed the arachnoidal prolongations accompanying the cranial nerves. It was transported along the olfactory nerve to the nasal cavities from which it reached the cervical lymphatics. From the perilymphatic spaces of the optic nerves, he found the thorotrast appearing in the lymphatics of the eyeball from which it continued along the perivascular lymphatic vessels of the angular and facial vein to the lymph nodes of the lower jaw and neck. Finally, the perilymphatic spaces of the inner ear were outlined, as well as the cranial nerves running out of the skull and the spinal nerves to the proximity of the ganglia.

Wustmann found that the soft meninges on the surface of the brain and in the depths of the fissures and convolutions constituted a protective filter against these particles. Only when a break in the barrier

between the fluid and brain tissues occurred through meningeal lesion could thorotrast find conditions favorable for penetration into the depths of the brain.

Because of the high specific gravity of thorotrast, he observed that it had a tendency to collect in the basal cisterns (39).

Such findings were in agreement with those of Radovici and Meller. The experimental results of these investigators, however, proved that the elimination of thorotrast was insignificant. Several months after injection the inert colloidal substance was still found in the process of being phagocytized. In other parts of the brain surface only a layer of cells stuffed with colloidal granules more or less thickened was found. Because of the accumulation of cells containing thorotrast, the thickness of the pia mater was found to be increased. It was evident that the retention of the substance resulted in a meningeal reaction with mobilization of the macrophages destined to phagocytize the foreign material. Even a year after encephalography performed with thorotrast on a monkey x-rays were still quite comparable to those first taken (26).

In view of their findings of flocculation of thorotrast over a period of twelve hours in dilutions greater than one in eight, and because of various sequelae occurring in patients in whom they used the colloid, Radovici and Meller felt that thorotrast could scarcely be considered as the ultimate substance for clinical application (26).

Injecting thorotrast into the perineural spaces Löhner and Jacobi demonstrated roentgenologically the outline of the large nerve trunks (18).

By means of the intraspinal injection of thorotrast, they were able to follow the course of the colloid from its collection at the lower lumbar sac and spinal subarachnoid space along the perineural fluid spaces towards the lymphatic channels into the prevertebral ganglia, and under the roots of the nerves. In this way, the cauda equina, the conus terminalis and the nerve roots were shown almost in a plastic manner by X-ray. Even though their injection of thorotrast was intraspinal, of course, the possibility that a variable amount of the colloidal substance might have become subdural is not unlikely.

They were also successful in demonstrating the sciatic plexus, the nerves of the gluteal group, and the sciatic nerve. It was felt that

a valuable development in spinal topographical diagnosis had been obtained (18).

Encouraged by such findings, they next injected a few cc. of thorotrast into an enlarged fluid-containing prolapse of a one month post-operative case of a left frontal lobe tumor. In the course of a day the substance spread out over the entire ventricular system. With the storage of the thorium dioxide along the ependymal membrane of the ventricular wall, the outlines of the lateral ventricles were clearly demonstrable. Moreover, the optic recesses and infundibulum, as well as the superior and inferior pineal recesses were nicely visualized (14).

Thereafter they used the thorotrast in cisternal injections and succeeded in outlining in patients by means of X-ray, the cerebral and cerebellar hemispheres, the important contours of the pons, the fissure of Sylvius and the orbits. Their findings in this regard were quite in accord with those of Radovici and Meller, and Wustmann (14).

In their experiments with encephalography Löhr and Jacobi found considerable elevation of lactic acid and reducing substance in the fluid spaces, even with thorotrast, "1073 D". They were instrumental in the production of "1093", which decreased the reducing and lactic acid forming qualities and also lowered the protective colloid quantity some 40 per cent (14).

After the injection of the new preparations, "1073 D" and "1073 F", "1093" and "1099", however, the presence of an acidosis in the fluid spaces was still confirmed.

Because such acidosis was often found to result in the production of pathological protein bodies, which lead to the precipitation of the colloid, they felt the preparation was still not suitable. Such precipitation would hasten the development of hydrocephalus which these investigators were able to demonstrate in a preparation given them by Wustmann.

It was their feeling that thorotrast was still not advisable for general clinical examinations, and that ventriculography with air remained the diagnostic method of choice for tumors of the brain (14).

In view of the miscibility of thorotrast with all body fluids, for the reason that its high specific gravity allows access to the recesses of the ventricular system, its great radiopacity requires relatively small

amounts of material and because fluid replacement of the cerebrospinal fluid is productive of fewer untoward symptoms, Freeman, Schoenfeld, and Moore have recently advocated thorotrast for ventriculography (11).

Besides Freeman, Schoenfeld and Moore, who have recommended thorotrast for ventriculography after their experience with it in twenty patients, Twining and Rombotham have also been enthusiastic after using it ventricularly in two cases (34).

According to Freeman and his collaborators, from the standpoint of roentgenography the ventricularly injected material normally leaves the cranial cavity within four hours, most of it presumably being absorbed into the blood stream by the arachnoid villi.

Radovici and Meller felt they had been able to confirm the passage of thorium dioxide into the circulation of certain animals by the exaggeration of the normal spleen and liver shadows in X-rays taken about twenty to thirty days after the suboccipital injection. Because of their findings of considerable quantities of thorotrast in the subarachnoid spaces as long as nine months after injection, they concluded that any such elimination must be slight (26).

Radovici, Bazgan and Meller came to the conclusion that although it was quite probable a very small quantity of thorotrast passed out into the circulation by the blood capillaries during the first days after the suboccipital injection of thorotrast, the greater part remained and was deposited slowly in the cells of the meninges (27).

After eliminating all technical sources of error, Wustmann was able to demonstrate thorium chemically in the sinus blood within the first three minutes after cisternal injection of the colloidal thorium dioxide. That such elimination was slight, was confirmed by his histological findings of thorotrast at the base of the brain in the conformation of the ventricular recesses, and in the subarachnoid spaces of the monkey which one and one-half months previously had received a ventricular injection of 3.5 cc. of thorotrast (39).

Freeman's supposition that ventricularly injected thorotrast is mostly absorbed into the blood stream by the arachnoid villi is therefore not in agreement with the experience of other investigators.

In 1933 Coe, Otell, and Hedley (4) injected neoskiodan into the suboccipital spaces of two anesthetized monkeys, but were unsuccessful in visualizing the subarachnoid spaces with this substance.

They next carried out similar experiments with thorotrast. In view of their experience with the cisternal injection of thorotrast in monkeys, they came to the conclusion that the advantage of thorotrast over air was not sufficient to warrant the use of such a hazardous procedure.

In 1935 Capua (3) reported numerous changes in the arachnoid and pia mater of a dog which died twenty days after the cisternal injection of 4 cc. of thorotrast. He found similar alterations in a patient that received thorotrast intraspinaly and died subsequently from a pancreatic malignancy.

Alexander, Jung and Lyman (2) found that the injection of colloidal thorium dioxide into the brain of dogs produced a relatively similar reaction to that evoked by olive oil or 40 per cent iodized poppy seed oil. Their examinations showed that the colloidal thorium dioxide acted as a foreign substance in the parenchyma of the brain.

Of importance in this regard because of similar pathological possibilities intracranially has been production of scar tissue by the subcutaneous injection of thorotrast experimentally and accidentally clinically.

In 1934 Boeminghaus injected 1 cc. of thorotrast subcutaneously to determine the reaction occurring over a long period of time. At first the area of injection remained painless. After several weeks, however, a firm infiltration developed, which increased in size slowly, and from which after several months time one could produce considerable pain in the affected extremity with movement or pressure. An X-ray a year later still showed the thorotrast. Excision of the area revealed that the loose subcutaneous tissue had changed into solid scar tissue which creaked under the section knife. In view of such findings, Boeminghaus felt that one could no longer say that the presence of thorotrast was an indifferent affair.

A similar example was found in the cases recently reported by Fleming and Chase in which there occurred an extravasation of thorotrast into the surrounding tissues when the substance was being given intravenously for the diagnosis of a liver condition. The cubital fossae became red and swollen. In about twenty-four hours these signs of inflammation subsided leaving a few small, firm lumps which the patient could feel under his skin. These remained un-

changed for a month, and then a gradual progressive enlargement was noted. Four months later, the masses in both arms were associated with considerable pain and interference with function.

On examination, the right antecubital fossa contained a series of firm nodules ranging from the size of a walnut to a pea, apparently in the subcutaneous tissue, and attached to the flexor muscles. In the left antecubital fossa were found similar smaller pea-sized nodules. All were dissected out and a Thiersch graft done on the right arm to restore the skin defect.

These nodules were difficult to section. Microscopically a large amount of pale, brownish refractive material was found lying partly free, but mostly within large, angular shaped cells, evidently of the macrophage type, which had phagocytized the material. The reaction appeared to be a relatively mature form of hyaline fibrosis.

As Fleming and Chase have pointed out, the proliferative reaction to thorotrast in the two cases examined by them was greatest in situations in which connective tissue is normally most abundant.

In 1932 Dixon and Heller (9) pointed out that the intracisternal injection of kaolin produced a steady increase of the arterial pressure, and it was felt by them that the hypertension resulted from the finely divided kaolin preventing the absorption of the cerebrospinal fluid. They also found rather consistent evidence of hydrocephalus in the experimental animals which were sacrificed at one, twelve, and twenty-seven month periods.

Heller found a similar cerebral hypertension with the injection of thorotrast, and on the basis of his results warned against the clinical application of colloidal substance for encephalography (9).

With such experimental observations in mind, we were able to emphasize certain important contraindications to the application of thorotrast for encephalography and ventriculography (33). Although as far as the X-ray demonstration is concerned, it is perfectly true that the substance leaves the ventricles within a period of four hours, it does not disappear from the cranial cavity, but, as Wustmann demonstrated in 1932, flows into the basal cisterns and spreads out into the subarachnoid space. In our monkeys, after the ventricular injection of thorotrast the X-rays revealed the disappearance of this material from the ventricles, occurring within half an hour. X-rays

taken three hours after ventriculography demonstrated an almost complete disappearance of the substance from the ventricles. With this disappearance from the ventricles, the opacity of the subarachnoid spaces and particularly the basal cisternae became more pronounced.

The retention of the granules of thorotrast in the meshes of the arachnoid network is in accord with the observations of Quincke and of Sicard and Cestan with dyes, and with those of Weed and others with lampblack.

X-rays of post-mortem serial sections from the neck to the pelvis taken some months after thorotrast ventriculography on a monkey showed the opacity of the thorotrast only about the spinal cord and none whatever in the lungs, spleen, or liver. This together with the fact that at autopsy examination there was no X-ray evidence of a significant elimination of thorotrast from the subarachnoid spaces following either ventriculography or encephalography represents fairly definite proof that no consequential transportation of the substance from the subarachnoid spaces into the blood stream occurs. This agrees with the work of Wustmann, Radovici, and Meller (26).

Microscopic sections of the monkey's brain ventricularly injected with thorotrast a few months previously revealed the presence of macrophages loaded with thorotrast in the meninges, where penetration of the substance into the depths of the brain was seen to be prevented by the protective barrier formed by the pia mater.

The similar presence of thorotrast within macrophages was observed along the ependymal wall of the lateral ventricle and in the perilymphatic spaces of the inner ear. Macrophages containing thorotrast were likewise shown in the perineural spaces of the optic nerve.

Not only are such findings in agreement with those of Wustmann, Radovici, and Meller, but from a consideration of Quincke's studies (29, 35) with cinnabar in 1872, such a distribution of thorotrast throughout the subarachnoid space could have been readily predicted.

As Weed mentions, the connection between the subarachnoid space and the inner ear has been generally accepted for years (36).

Thorotrast was not visualized in the cervical lymph nodes of any of our monkeys which had ventricular injections, which suggests that most of the outlining of these nodes was the result of the extravasation

of thorotrast into the subcutaneous tissues during the cisternal injection, such as occurred not infrequently in our cisternally injected cats. Essick, Kubie, and Schultz (38) emphasize the fact that it is extremely difficult to perform a lumbar or occipital puncture of the meninges without getting seepage of a solution or a suspension into the tissues surrounding the dural puncture with subsequent coloration of the regional lymphatics, and lymph nodes, an observation quite in agreement with our own experience.

Interestingly enough, in this regard Weed found that when suspensions of carbon particles were used with low pressure, no granules were found in the cervical nodes. When, however, the injection of India ink was made at a high pressure, 100 mm. of Hg, the cervical nodes were found to contain carbon particles. This he explains as probably being due to the breaking down of the delicate filters, which ordinarily hold back the granular substances and maintain intact the cerebrospinal fluid spaces (36).

Injection of thorotrast in the presence of an increased intracranial pressure would undoubtedly result in a similar course of the material.

As far as a colloidal material such as thorotrast is concerned, we found that even high pressures were not successful in transporting the material either from the subarachnoid space to the peripheral spinal nerves or from the peripheral nerves to the subarachnoid space of the spinal cord. That a sufficiently high pressure of sufficient duration would accomplish the passage of the barrier goes without saying, but as far as normal pressure relationships are concerned, it seems rather obvious that there is no free flow of thorotrast between these two regions.

This agrees with the experience of Quincke (29) with cinnabar, Ianow and Romodanowsky (13) with specially prepared suspensions of India ink and various colloids, and with the recent investigations of Abel and his collaborators.

As might have been expected from the already mentioned work of Dixon and Heller (9) and from the observations of Löhr and Jacobi (14), we were successful in producing hydrocephalus with cisternal and ventricular injections of this material in our animals.

The hydrocephalus was fully as pronounced in a cat which received one subarachnoid injection of 1 cc. of thorotrast as it was in those

which received both cisternal and ventricular injections of 1 cc. amounts of thorotrast.

In those cats which received a cisternal injection of thorotrast, hydrocephalus, though not pronounced, was evident within a period of two months provided the subarachnoid space had not been incompletely filled at the time of the injection.

As long as sufficient material had been used for a diffuse though slight outlining of the subarachnoid space as observed from X-rays of the removed brain, hydrocephalus occurred within this short period of two months. Three dogs developed hydrocephalus within two months after ventricular injections of 2 cc. in one animal and 1 cc. in two of the other animals. Two monkeys receiving three ventricular injections totalling 4.5 and 4 cc. of thorotrast respectively over a period of three months developed fairly marked degrees of hydrocephalus. Had a longer period of time for observation of these animals been available, the extent of their hydrocephalus probably would have been more pronounced.

As far as the comparative doses of 1 to 2 cc. of thorotrast in the experimental animals with the 6 cc. used by Freeman, Schoenfeld, and Moore in patients is concerned, the former represents of course a greater proportionate amount. Doses of less than 1 cc. of thorotrast did not however, give satisfactory visualization in the animals.

Another point of interest in this respect to which we have already alluded is the finding of precipitation of the thorotrast in dilutions with spinal fluid above 1 in 8. In the use of thorotrast for ventriculography in patients this dilution is considerably exceeded, and the likelihood of a subsequent flocculation or precipitation therefore on this basis more probable. This suggests that the probabilities of an hydrocephalus with such 6 cc. doses over a period of time is not unlikely. Moreover, the previously mentioned findings of Boeminghaus and of Fleming and Chase of the production of scar tissue following the subcutaneous injection of thorotrast experimentally and accidentally clinically, leads to most interesting speculation concerning a similar effect in the meningeal spaces of the brain. In their experience this condition developed over a period of several months and this proliferative reaction to thorotrast was found to be greatest in those situations in which the connective tissue was normally most abundant.

The possibility of the formation of such scar tissue in the meningeal spaces over a period of months makes the likelihood of a future hydrocephalus the more probable, and also raises the question of the development of epilepsy from such cicatrices.

Our findings were in agreement with those of Radovici and Meller, and others, who noted that the long stay of the colloidal thorium dioxide on the surface of the central nervous system was productive of an aseptic meningeal reaction with mobilization of the macrophages destined to phagocytize the foreign material.

Freeman and his associates report two deaths and two severe reactions in their series of twenty cases, and they mention that the greatest danger in the use of the method seems to lie in the inflammatory effects in cases in which the ventricular system is obstructed.

In spite of any claim to mildness of reaction following ventriculography with thorotrast, the obvious aseptic type of meningeal reaction it provokes produces an increase of pressure which with any obstruction to the ventricular system would produce symptoms unless relieved by ventricular tapping and operation.

One of our monkeys which received a ventricular injection of 6 cc. of thorotrast died within thirty-six hours, so that there seems to be a definite limit to the amount of the material that can be injected with any immediate degree of safety.

The radioactivity of thorotrast is a well established fact. We were able to demonstrate this property by autohistioradiographs developed from the paraffin blocks from which histological sections of one of the monkey's brains ventricularly injected a few months previously with thorotrast had been taken. This was a method very similar to that used by Martland in 1926.

From the standpoint of radioactivity, its particular importance intracranially is evident in its intensely localized effect, and its wide distribution in the subarachnoid space in the proximity of the important structures of the central nervous system.

There is little doubt that ventriculography with thorotrast is productive of a spectacular delineation of this system. On the other hand, as Dandy (8) has emphasized, "It has been stated by enthusiasts for the use of liquids producing opaque shadows (such as thorium dioxide and iodized poppy-seed oil) that the employment of these solutions is especially advisable in the differentiation of tumors in

this region (third ventricle) because ventriculography with air is ineffective. Such a statement is only an admission that the injection of air was not properly made. There is no tumor of the brain causing intracranial pressure, and this includes tumor of the pineal body and tumor of the third ventricle, that cannot be accurately localized by ventriculography. There is never, therefore, in my opinion, justification for the use of opaque material as a means of diagnosis."

In conclusion it is our opinion that in view of the more or less permanent retention of thorotrast in the subarachnoid spaces, affording the possible development of cicatrices, its presence in the optic and other cranial nerves, as well as its location in the internal ear, with the obvious opportunity for radioactive effect here and throughout the subarachnoid spaces, and because of its partial obstruction to cerebrospinal fluid absorption with resultant hydrocephalus, that the material should be unequivocally condemned for encephalography or ventriculography.

SUMMARY

1. In reviewing the literature concerning the clinical use of thorotrast for diagnostic purposes in various organs of the body, one invariably discovers that an initial enthusiasm is replaced by a condemnation of this substance by most investigators.

2. The clinical importance of the radioactive effect of thorotrast has been emphasized in the publications of Flinn and Martland. In the usual external application of radioactive materials the alpha ray, responsible for about 95 per cent of the total energy emitted by radioactive substances is unimportant because it is filtered out easily and its range is short. The storage of radioactive materials internally, however, such as occurs after an intravenous or cerebrospinal injection of thorotrast, provides a continuous opportunity for the damaging effect of the alpha radiation not previously experienced. Biologically these alpha rays are more destructive than either the beta or gamma rays, and their activity is intensely localized.

3. After the intravenous injection of thorotrast, various reactions have been reported. These have included slight to moderate febrile manifestations, vomiting in hepatic cirrhosis, sometimes lasting several days, anaphylactic phenomena with particularly severe asthmatic

attacks in individuals so disposed, and a few cases resulting in purpuric manifestations.

4. The elimination of thorotrast after intravenous injection has been found to be insignificant by numerous investigators. Moreover, after ventriculography with thorotrast, although we have found that the ventricular opacity disappears within a period of four hours, the material has not been eliminated from the cranial cavity but has become indefinitely stored in the subarachnoid space.

5. In hepatolienography stages between an early hepatitis and the beginning of an experimental cirrhosis have been reported as well as variations from acute splenic tumor to splenic rupture with necrotic changes. Impairment of hepatic function and arrest of phagocytic activity has likewise been noted.

6. Ebhardt emphasized that regardless of any question of a damaging effect of thorotrast, a negative result from hepatolienography does not with certainty eliminate the existence of small metastases. Conversely, the positively appearing x-rays are often subject to numerous sources of error.

7. It is our opinion that if after due consideration of the various clinical and experimental results of thorotrast hepatolienography, one weighs the necessity and diagnostic value of the procedure against the possibilities of damage both immediate and remote, one can hardly become enthusiastic about its clinical application.

8. Menville and Ane noted such extreme lymphatic destruction after injections of thorotrast that they speculated enthusiastically upon the use of thorotrast in the metastatic involvement of lymph nodes, and felt that it might be equivalent to a surgical removal.

9. The experimental work of Bennett revealed the inadvisability of using thorium dioxide in cases of tuberculosis because of the rapid dissemination of the disease through the tissues following the injection of this material.

10. Because of the frequency of abortion, and for the reason that the contraindications far outweigh any beneficial diagnostic possibilities, the clinical application of thorotrast for placentography has not met with any enthusiasm.

11. Because of frequent indefinite retention of thorotrast in the kidney pelvis and a similar storage of the material in the kidney paren-

chyma by means of pyelovenous transportation, as well as a not infrequent ureteral blockage by precipitated thorotrast, the general opinion of most investigators is that pyelography with thorotrast is definitely contra-indicated.

12. The procedure of cerebral angiography, particularly for those not accustomed to its use, possesses technical difficulties, and is one not exempt from danger. It is difficult to believe that many cerebral neoplasms can ever be localized by an arterial change even if perfect injections were possible. Moreover, cerebellar tumors and vascular abnormalities, being outside the limits of carotid circulation, would appear to be precluded from diagnosis unless the vertebral arteries were also injected. If, after due consideration of the potentially damaging effects of thorotrast, there is any justification for its arterial injection, this would seem to be reserved for the diagnosis of vascular abnormalities in properly selected cases.

13. Because of the indefinite retention of thorotrast in the subarachnoid spaces, affording the possible development of cicatrices, its presence in the optic and other cranial nerves, as well as its location in the internal ear, with the obvious opportunity for radioactive effect here and throughout the subarachnoid spaces, and in view of its partial obstruction to cerebrospinal fluid absorption with resultant hydrocephalus, we feel that the material should be unequivocally condemned for encephalography or ventriculography.¹

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¹ We are indebted to Dr. W. V. Cone whose interest in thorotrast was largely respon-

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THE PATHOLOGICAL PHYSIOLOGY OF CHRONIC CARDIAC DECOMPENSATION

M D ALTSCHULE, M D

From the Medical Service and Medical Research Laboratories of the Beth Israel Hospital and the Department of Medicine, Harvard Medical School, Boston

CONTENTS

Introduction	76
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Part One

I Cardiac Output, Variations in Response to Therapy	76
II Pulmonary Physiology	87
III Pulmonary Circulation Time	89
IV Reflexes from the Lungs	90
V Arterial and Venous Blood Oxygen Arteriovenous Oxygen Difference Tissue Oxygen Tension	91
VI Alveolar Air and Arterial and Venous Blood Carbon Dioxide Carbon Dioxide Dissociation Curves Arterial and Venous Blood pH	93
VII Blood Lactic Acid and Oxygen Debt	97
VIII The Venous Pressure	99
IX Cerebrospinal Fluid Pressure	100
X Plasma and Edema Fluid Proteins	101
XI Blood Volume and Viscosity Hemoglobin and Erythrocyte Count	103
XII Peripheral Capillaries	105
XIII Lymphatics	105
XIV Temperature Regulation Insensible Perspiration	106
XV Basal Metabolic Rate	107
XVI Liver Function	107
XVII Renal Function	109
XVIII Gastro Intestinal Function	111
XIX Creatine Metabolism	111

Part Two

A Edema	112
B Cyanosis	118
C Dyspnea	120
D Orthopnea	124
E Effect of Therapy on Signs and Symptoms and on Cardiovascular Physiology Status of the "Adequately" Treated Cardiac Patient.	129
F "Backward" Failure "Forward" Failure A Generalization Concerning the Pathogenesis of the Signs and Symptoms of Congestive Failure	134

INTRODUCTION

Dyspnea, orthopnea, cyanosis, venous engorgement, and edema were well known to the clinicians of a century and more ago as evidences of heart disease. During the last twenty-five years intensive study of these manifestations of cardiovascular disease by physiological and biochemical methods has resulted in a large volume of data bearing on the mechanisms underlying the production of these and other signs and symptoms of cardiac decompensation. It is the purpose of the present communication to review and to analyze critically these data and to evaluate their significance in understanding the pathogenesis of cardiac decompensation. The need for a review of this sort is greater than is at first apparent. While a number of monographs on the physiology of congestive failure have appeared during the past decade, almost all have been written in order to support or criticize various theories of the pathogenesis of heart failure. In the present work theoretical considerations will be minimized.

Only the physiological manifestations of chronic congestive failure will be treated here; paroxysmal dyspnea and anginal pain and the complications which occur in failure, such as embolism, chronic pulmonary disease, etc. will not be discussed.

This presentation is divided into two portions (*a*) analysis of the physiological measurements of cardiac decompensation as such, and (*b*) the interpretation of this physiological knowledge in explaining the meaning of the cardinal signs and symptoms of congestive failure: dyspnea, orthopnea, edema and cyanosis. The conclusions reached cannot be regarded as final in any sense; they represent merely an attempt to describe the pathogenesis of the principal signs of cardiac decompensation in the light of what has thus far been observed in laboratory studies.

PART ONE

I. CARDIAC OUTPUT

Discussion of the pathological physiology of cardiac decompensation properly begins with consideration of the output of the heart. The great bulk of the reported data concerns the findings in cardiac

patients under basal conditions or at least at rest. Such data, while of considerable value, give no information as to the cardiac output at a time when patients experience most or all of their symptoms, i.e., during exercise. The few reported determinations in cardiac patients during or shortly after exercise (223) (313) (13) (14) (242) are of extremely doubtful accuracy for reasons which will soon become apparent.

Another source of confusion arises from the multiplicity of the methods used. The reader is referred to Grollman's (130) book for a full description of the various methods which will here be treated only briefly. The Fick principle, which states that cardiac output in cubic centimeters equals oxygen consumption of the body in cubic centimeters per minute divided by arteriovenous oxygen difference per cc. is the basis of most of the methods employed.

Estimation of oxygen consumption can be accomplished by means of direct, simple and accurate methods. Determination of the arteriovenous oxygen difference, however, is beset with difficulty. Arterial blood can be secured with ease; the same holds for alveolar air which is in equilibrium with it. Mixed venous blood, on the other hand, is obtainable only from the right auricle or ventricle; accurate estimation of the arteriovenous oxygen difference by direct methods is, therefore, almost impossible in the clinic. A variety of methods for the indirect estimation of arteriovenous oxygen difference have consequently been developed. For technical reasons certain authors have preferred the use of carbon dioxide or various foreign gases instead of oxygen in measuring the cardiac output in man. For oxygen consumption and arteriovenous oxygen difference in Fick's formula may be substituted corresponding figures for any foreign gas or for carbon dioxide production and arteriovenous difference. The technique of the method depends on the gas employed. Methods employing the Fick principle may be divided into four main groups:

(a) *Cardiac Puncture.* In this method oxygen consumption is measured by the usual procedures. Cardiac puncture is employed to secure mixed venous blood from the right auricle, arterial blood being obtained from a convenient artery. The applicability of this procedure is of course limited. No data obtained by this method in

patients with chronic congestive failure are at present available in the literature.¹

(b) *Analyses of Arterial and Peripheral Venous Blood.* Here again oxygen consumption is estimated by the usual methods. Blood samples taken usually from the femoral or radial artery, and the antecubital vein are analyzed for oxygen or carbon dioxide in order to determine the arteriovenous difference. While the use of blood from any artery is valid, since arterial blood throughout the body is of uniform composition, use of venous blood from a single portion of the body, such as the arm, leads to error since it differs from mixed venous blood. Results obtained by such methods may indicate trends in the cardiac output, but general concepts based on such data must be accepted with reservations.

(c) *Rebreathing Methods.* These may be divided into two groups:

(1) Methods in which rebreathing is used to secure air in equilibrium with mixed venous blood for measurement of oxygen or carbon dioxide content. These methods have been largely discarded for technical reasons.

(2) Methods using a foreign gas. These include the Krogh-Lindhard nitrous oxide method (130) and the Grollman acetylene method (130). The last in a modified form (131) is the method of this type most widely used at present. These methods employ measurements of rate of absorption of a foreign gas by the blood in the lungs during a period of rebreathing and are based on the principle that the amount of a gas so absorbed in a given time is governed by the volume of pulmonary blood flow. The entire period of rebreathing must be short enough so that no appreciable recirculation of blood once exposed to the air in the rebreathing bag can occur; it is clear, therefore, that no acceptable determinations can be made during periods of rapid circulation, i.e., exercise. Changes in circulation time due to variations in the condition of patients with congestive failure might also introduce errors. Another source of inaccuracy lies in the fact that

¹A paper containing such data appeared after this review was set up in type (McGuire, J., Hauenstein, V., and Shore, R. Cardiac Output in Heart Disease Determined by the Direct Fick Method, Including Comparative Determinations by the Acetylene Method. Arch. Int. Med. 60, 1034, 1937). The findings of these authors are in accord with the views expressed in this review.

it is difficult to obtain thorough mixing of the gas mixture being rebreathed in patients with changes in the lungs due to congestive failure (260) (311). It is, therefore, necessary to prolong the re-breathing period, thereby increasing the possibility of recirculation of blood. Another source of inaccuracy in the Grollman acetylene method is the fact that small errors in analysis lead to large errors in results; duplicate determinations within less than plus or minus ten per cent may not be obtained. This twenty per cent error may completely mask some of the changes which occur in response to therapy.

(d) *Ethyl Iodide Methods*. In these methods ethyl iodide consumption and arteriovenous difference are substituted for oxygen consumption and arteriovenous difference in Fick's formula. The original ethyl iodide method of Henderson and Haggard (155) yielded erroneous results because these authors disregarded the presence of ethyl iodide in the venous blood and incorrectly estimated the partition coefficient of that substance between air and blood. These errors were, however, constant, so that many authors believed that the method was useful. Starr and Gamble (318) revised the method eliminating many of the errors. The validity of this new method in exercise has not, however, been established.

Results obtained in congestive failure with all methods based on exchange of gases in the lungs have been criticized on the basis of the known impairment of pulmonary function in cardiac decompensation. A method which avoids this criticism is one in which the cardiac output is calculated from the behavior of a dye injected intravenously (138). In this method, although the values obtained are probably not identical with the actual cardiac output, the errors are constant so that a figure in definite relation to the actual cardiac output is obtained. The fact that a method yields "comparative" and not "true" values is in itself of no great significance; the true value of the cardiac output is an interesting, but for the purposes of this discussion not an important, figure. Actually no method now available permits the estimation of the cardiac output with absolute accuracy. Some yield results which approach it more closely than others.

Whatever the criticisms which can be leveled at one method or another, valid conclusions as to changes in cardiac output can be

drawn if all the diverse methods yield essentially similar results. Such is fortunately the case in cardiac decompensation.

Many authors who have studied the blood gases in congestive failure have obtained data on the arteriovenous oxygen or carbon dioxide difference. These data are of the same significance as measurements of the cardiac output itself, for according to Fick's formula,

$$\text{cardiac output} = \frac{\text{oxygen consumption}}{\text{arteriovenous difference}}$$

Observations on Cardiac Output in Congestive Failure. The following authors have reported a low cardiac minute volume output or a high arteriovenous difference compared to the normal in cardiac failure.

It is true that the cardiac output in occasional patients with heart failure is of the same magnitude as that of some normal individuals. That this overlapping of values occurs is not surprising, for the normal minute volume output of the heart, according to Grollman (130), varies between 1.9 and 2.5 liters per square meter of body surface per minute, a range of about thirty per cent. Donal, Gamble, and Shaw (77) found a somewhat greater range of variation. It is apparent that while some normal individuals have a cardiac index (cardiac output per square meter of body surface per minute) of 1.9, this level might very well represent cardiac decompensation in a patient whose normal value is 2.5.

Patients with heart disease, but without evidences of failure, usually have a normal minute volume output; in occasional instances some decrease in cardiac output occurs before signs of failure appear.

Eppinger, and his coworkers (85) (86), and Harrison and his coworkers (144) found a normal or increased cardiac output in heart failure. The experiments of the first have been criticized on the basis of faulty technique (130) and are now thoroughly discredited. The results of the latter, on closer examination, are found to conform to those of the majority of workers in this field. Of nineteen patients with moderate or severe congestive failure, ten had values for cardiac output within or only slightly below normal limits. Of these ten, however, nine had basal metabolic rates ranging from plus 15 to plus 78 per cent. Obviously the cardiac output in such cases cannot be compared with that of undecompensated individuals in whom the

TABLE I

YEAR	AUTHOR	METHOD
1915	Means and Newburgh (225)	Blood gas analysis
1916	Lundsgaard (205)	Krogh-Lindhard
1917	Pearce (255)	Blood gas analysis
1918	Lundsgaard (206) (207) (208)	Blood gas analysis
1919	Sonne and Jarlov (315)	Blood gas analysis
1919	Hnrrop (152)	Blood gas analysis
1921	Barcroft, Bock, and Roughton (18)	Modified Plesch
1923	Stewart (324)	Blood gas analysis
1923	Carter and Stewart (54)	Blood gas analysis
1923	Meakins, Dautrebande, and Fetter (223)	Meakins and Davies
1924	Grant (126)	Blood gas analysis
1925	Dieuaide (76)	Blood gas analysis
1925	Rabinowitsch (274)	Blood gas analysis
1925	Henderson and Haggard (155)	Original ethyl iodide
1926	Dautrebande (72)	Meakins and Davies
1926	Bielschowsky (29)	Original Grollman
1926	Mobitz (233) (234)	Original ethyl iodide
1927	Mobitz (235)	Original ethyl iodide
1928	Kininmonth (183)	Original ethyl iodide
1929	Weiss (344)	
1929	Lauter and Baumann (197)	Original ethyl iodide
1930	Ringer and Altschule (282)	Original ethyl iodide
1930	Weiss and Ellis (345)	Original Grollman
1930	Kroetz (188)	Original Grollman
1930	Alt, Wnlker, and Smith (3)	Bock and Field
1930	Smith, Walker, and Alt (313)	Bock and Field
1930	Bansi and Groscurth (13) (14)	Original Grollman
1931	Ewig and Hinsberg (91)	Modified Plesch
1931	Harris and Lipkin (140)	Blood gas analysis
1932	Jansen, Knipping and Stromberger (173)	Blood gas analysis
1932	Stewart and Cohn (326)	Original Grollman
1932	Grossman and Herzog, (132)	Original Grollman
1932	Hnmlton, Moore, Kinsman, and Spurling (138)	Dye (138)
1933	Starr, Collins, and Wood (316)	Modified ethyl iodide
1933	Nylin (211) (242)	Original Grollman
1933	Kinsman, Moore, and Hamilton (185)	Dye (138)
1934	Starr, et al. (317)	Modified ethyl iodide
1935	Friedman, Clark, Resnik, and Harrison (110)	Modified Grollman
1935	Weiss and Ellis (346)	Blood gas analysis
1935	Starr and Gamble (319)	Modified ethyl iodide
1935	Kinsman and Moore (184)	Dye (40)
1936	Kerkhof (179)	Original Grollman
1936	Goldbloom (122)	Original Grollman
1936	Altschule and Volk (9)	Modified ethyl iodide
1936	Stewart (325)	Modified Grollman

basal metabolic rate is within limits of plus or minus ten per cent of the standard average normal. The importance of the relationship between the cardiac output and oxygen consumption has been stressed by the author (9) recently. It is apparent that the cardiac output *in proportion to oxygen consumption* is low in Harrison's patients with cardiac decompensation. In general, therefore, it must be concluded that a diminution in cardiac output relative to the metabolic requirements of the body is the rule in congestive failure.

The diminution in cardiac output per beat is usually greater than in cardiac output per minute, since the heart rate is generally accelerated with congestive failure. Even more marked deviations from the normal are found if the cardiac output per minute or per beat is compared to the heart size, as was pointed out by Nylin (211) (242) and by Starr and Gamble (316) (317) (319) and their coworkers.

Although no trustworthy measurements of cardiac output during exercise in congestive failure exist, indirect evidence as to changes in cardiac output is available. Measurements of the oxygen debt in patients with cardiac failure show an increased debt after exercise (see below). Similarly, the venous pressure shows a greater rise after exercise in cardiac patients than in normal individuals (145) (299). These facts suggest that in cardiac decompensation the output of the heart does not increase normally in response to work.

It has been shown (7) that even a small increase in venous pressure in normal individuals results in striking increases in cardiac output. Patients with high venous pressure due to congestive failure fail to exhibit this response.

The low cardiac output found in congestive failure may be due to at least one of three causes. It has been shown (54) (76) (316) (317) (319) that rapid heart action in itself reduces the minute volume output. In paroxysmal tachycardias the rapid heart action is usually the sole factor in diminishing the output of the heart. Similarly, auricular fibrillation in itself may cause a fall in cardiac output (179) (223) (313) (324). However, in by far the greatest number of patients with heart failure, the diminution in output is mainly or solely the result of weakness of the myocardium.

A decrease in the cardiac work occurs in heart failure since the work of the heart is directly proportional to the cardiac output. Numerous

saturation of the arterial blood, the cardiac output remaining the same (15). Similarly, edema may be caused to disappear by the administration of urea or ammonium chloride (281), theobromine derivatives (111) (326), or mercurials (111) without any change in the output of the heart. It is, therefore, not to be expected that clinical improvement should be accompanied necessarily by an increase in cardiac output.

Effect of Digitalis on Cardiac Output in Congestive Failure. Many studies of the output of the heart in decompensated patients following the administration of digitalis have been reported. The interpretation of much of this data, especially in its relation to clinical improvement is difficult, since, as noted above, clinical improvement may be unrelated to changes in cardiac output. The usual procedure has been to take decompensated patients immediately after admission to the hospital, measure the cardiac output, administer digitalis, and then measure the cardiac output again. However, such patients have also received the benefit of other therapeutic procedures, such as bed rest, sedation and limitation of fluids and salt; improvement in clinical status may be due to these, rather than the administration of digitalis itself. In order to control such experiments adequately, further studies of the cardiovascular system should be made after the withdrawal of the drug. This has been done systematically in only three series of experiments, those of Ringer and Altschule (282), Stewart and Cohn (326), and Starr et al. (320). Ringer and Altschule found that all of fourteen patients with auricular fibrillation exhibited an increase in cardiac output to or toward normal limits that could be definitely ascribed to digitalis but in only one of nine patients with regular rhythm did similar changes occur. Similarly, Stewart and Cohn (326) observed that all of three patients with auricular fibrillation showed an increase in cardiac output after digitalis; two of four with regular rhythm exhibited a similar response, a third showing a definite change the first time and a questionable one the second time the drug was given. Starr and his coworkers (320) noted significant increases in the output of the heart in decompensated patients with regular rhythm or auricular fibrillation; in the latter group of patients, increases in output per beat were much more striking than in output per minute. The results of Starr and his coworkers (320) are, how-

studies of the efficiency of the failing heart, i.e., relation of oxygen consumption to work performed, have been made on isolated heart or heart-lung preparations. The latest of these is that of Peters and Visscher (257). All authors agree that the efficiency of the failing heart is much reduced. Other studies of this subject on intact animals have been reported (146), but are open to severe criticism on technical grounds. It has been claimed (141) that diminished cardiac efficiency rather than low cardiac output determines the presence or absence of congestive failure. This probably is not the case. It is difficult to understand how edema develops in the ankles merely because the heart, located five feet distant, shows a low ratio of energy input to work output, i.e., low efficiency, in patients with cardiac decompensation. Changes in cardiac dynamics can influence the tissues only through the cardiac output and the venous pressure. It must be concluded that cardiac efficiency is interesting and important but is not directly responsible for the development of the signs and symptoms of congestive failure.

The severity of the signs and symptoms of cardiac decompensation does not parallel the decrease in cardiac output as was recently stressed by Harrison (141). The reasons why they do not, and indeed cannot, will be discussed in another portion of this review.

Effect of Therapy on Cardiac Output in Congestive Failure. Changes in cardiac output in response to therapy are difficult to evaluate. Some patients show improvement of clinical signs and symptoms while others do not, or even become worse, after administration of the same drugs; similarly the physiological status of some patients with congestive failure responds, while that of others does not, to the same therapeutic procedures. Therefore, complete uniformity as regards changes in cardiac output after the administration of drugs is not to be expected. Attempts have been made to correlate improvement in clinical status with changes in cardiac output. These, too, are not free from error, for fluctuation in the severity of symptoms, such as dyspnea, may be entirely unrelated to observable changes in cardiovascular or pulmonary dynamics. Even objective signs of cardiac decompensation may disappear without significant change in the output of the heart. Thus cyanosis is relieved by permitting patients to breathe air enriched with oxygen, which changes only the oxygen

saturation of the arterial blood, the cardiac output remaining the same (15). Similarly, edema may be caused to disappear by the administration of urea or ammonium chloride (281), theobromine derivative (111) (326), or mercurials (111) without any change in the output of the heart. It is, therefore, not to be expected that clinical improvement should be accompanied necessarily by an increase in cardiac output.

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II. PULMONARY PHYSIOLOGY

The functions of the heart and of the lungs are intimately related; the earliest clinical evidences of congestive failure are usually manifestations of altered pulmonary physiology. Many observers have noted an abnormally large respiratory minute volume in patients with cardiac decompensation after exercise (25) (53) (145) (148) (156) (177) (252) and during rest (25) (50) (51) (76) (145) (148) (149) (173) (178) (186) (250) (252) (254) (280). Respiration is shallow, but the rate is greatly increased so that the minute volume is greater than normal. In general, the increase in respiratory minute volume is associated with dyspnea or the sensation of shortness of breath in individual patients but it does not itself explain dyspnea; normal individuals in whom increases of respiratory minute volume comparable to those observed in congestive failure are induced by exercise or inhalation of carbon dioxide, do not become dyspneic (84) (149) (254). Cardiac patients with more severe degrees of dyspnea usually have a larger respiratory minute volume than those in whom dyspnea is less marked.

Measurements of the total lung volume and its subdivisions have been made in patients with congestive failure for over a quarter of a century. The validity of most of the earlier methods has been questioned by Christie and Meakins (58). However, the results obtained by all methods are in essential agreement qualitatively. The total lung volume has always been found diminished (30) (32) (173) (177) (209) (210) (221) (260) (291) (309). The mid-capacity is also decreased according to some authors (30) (260) (291) (309) (310) (311), though Bittorf and Forschback (32) found it relatively increased and Lundsgaard and Schierbeck found it variable (209) (210). The complementary air is likewise diminished (30) (32) (291). Many authors (30) (32) (47) (84) (173) (186) (221) (260) (291) (309) (310) (311) have noted that the reserve air is greatly diminished, often to the point of complete disappearance. Relative or absolute increase in the residual air has also been generally found (30) (32) (47) (173) (209) (210) (221) (260) (291) (309) (311). The measurement most frequently employed in studying patients with cardiac decompensation, however, is that of the vital capacity. Of all the subdivisions of the lung volume this is by far the easiest to measure. Low values

in congestive failure have been reported by all observers (18) (30) (32) (34) (35) (47) (53) (54) (84) (135) (149) (157) (177) (178) (184) (186) (217) (221) (250) (253) (254) (260) (274) (280) (282) (291) (311) (324) (345). For many years it has been customary to ascribe the dyspnea of heart disease to this reduction in vital capacity. As Christie and Meakins (58) have pointed out, however, it is not changes in vital capacity itself but rather in total capacity and reserve and residual air volumes which are responsible, at least in part, for dyspnea. In a general way, the deviations from the normal in the total lung volume and its various subdivisions including the vital capacity parallel the severity of the clinical condition. The importance of the vital capacity lies in the fact that it reflects changes in other, more important, but much less easily measured aspects of pulmonary physiology.

The mechanisms underlying these changes in pulmonary physiology have been elucidated, by means of experiments in animals, by Romanoff (284) and Drinker, Peabody, and Blumgart (80). These authors showed that pulmonary congestion prevents the entrance of the normal amount of air into the lungs because of encroachment by the engorged vessels on the air spaces; this finding supports Von Basch's earlier theoretical analysis of the pulmonary changes. Romanoff's (284) experiments further showed that increased rigidity of the lungs also occurred in pulmonary congestion. Decreased distensibility and elasticity has been demonstrated during life in patients with cardiac decompensation by Christie and Meakins (58) and Kaltreider and McCann (177); these changes paralleled the severity of the signs and symptoms of heart failure. In effect, the decreased distensibility and elasticity lead to a condition of the lungs closely resembling emphysema.

The intrapleural pressure was also measured by Christie and Meakins (58) in patients with cardiac decompensation and found to be less negative than normal. This in itself may interfere with normal pulmonary function. One cause of this abnormal intrapleural pressure is the state of apparent emphysema of the lungs described by these authors. Another causative factor is anoxemia, which has been shown by Prinzmetal, Lonergan, and Brill (272) to cause tachypnea and hyperpnea and hence an increase in trapeural pressure. In-

creased intrapleural pressure is recognized as an important cause of elevation of the peripheral venous pressure (58) (163).

In summary, the patient with severe congestive failure has lungs which contain less air than normal, have an increased dead space, and are unable to function properly because of diminished distensibility and elasticity of the lungs themselves and also because of decreased negativity of the intrapleural pressure.

Many attempts have been made to correlate changes in pulmonary physiology with the degree of dyspnea exhibited by patients with congestive failure. The most recent, that of Harrison (141) employs the ratio between respiratory minute volume and vital capacity as a measure of dyspnea. This does not appear to be valid. Dyspnea, being purely subjective, cannot be measured objectively. Most clinicians have at some time observed the marked disparity between the sensation of dyspnea and objective changes in pulmonary function in patients with heart disease. The author has observed striking changes in the degree of dyspnea in cardiac patients with absolutely no change in vital capacity or respiratory minute volume. The relation between pulmonary function and dyspnea will be discussed at greater length in another portion of this review.

III. PULMONARY CIRCULATION TIME

The significance of the pulmonary circulation time in health and disease has been discussed at length by Blumgart (33) in his review of the subject. All authors have observed slowing of the circulation in cardiac decompensation. Blumgart stressed the relation between that sign of pulmonary congestion and another, diminution of the vital capacity due to increase in the amount of blood in the lungs. In recovery from congestive failure both returned to or toward normal. He also pointed out that slowing of the circulation time occurs in myxedema; this was regarded as evidence not of pulmonary congestion but of diminution in the cardiac output. Altschule and Volk (9) have shown that in patients with hypothyroidism, prolongation of the circulation time parallels, in a general way in individual patients, reductions in the cardiac minute volume output. However, as these authors showed (9), equivalent reductions in cardiac output in patients with pulmonary congestion are associated with much greater

slowing of the pulmonary circulation time than in subjects without congestion. It may be taken for granted, therefore, that the slowing of the circulation time in the lungs in cardiac decompensation is due (1) to the lowered cardiac output which occurs in that condition and (2) to engorgement of the pulmonary vessels.

Slowing of the pulmonary circulation time, like diminution of the vital capacity, is frequently associated with dyspnea in patients with congestive failure. The measurement of the pulmonary circulation time is of use in distinguishing between dyspnea due to cardiac decompensation and dyspnea due to pulmonary disease.

IV. REFLEXES FROM THE LUNGS

Drinker, Peabody and Blumgart (80) in 1922 showed that when congestion of the lungs was induced by clamping the pulmonary veins, an immediate increase in respiratory rate occurred. Underhill (335) and Haggart and Walker (133) reported similar results when branches of the pulmonary artery were ligated, even though no change in cardiac output occurred. A marked increase in pulmonary arterial pressure was noted in these experiments. However, the reflex origin of the observed changes in respiratory rate was not established until 1929, when Churchill and Cope (59) reported experiments in which engorgement of the pulmonary vessels was induced in a lung completely isolated from the body except for its nerves. These observers clamped off the artery and vein to that lung and then injected varying amounts of fluid into the pulmonary vessels; rapid, shallow respiration invariably resulted which could be terminated by withdrawal of the injected fluid. It could also be prevented by section of the vagus nerve before the injection and terminated by cutting that nerve after injection. Similar experiments were reported several years later by Harrison, Calhoun, Cullen, Wilkins, and Pilcher (142) and by Harrison, Calhoun, Marsh, and Harrison (143).

It has been supposed for many years that the abnormal rigidity of the lung in congestive failure found at post mortem is a cause of dyspnea during life. Many authors have expressed the belief that this impaired collapsibility activates the Hering-Breuer reflex, thereby causing rapid shallow breathing. The validity of this concept, since it was based on post mortem findings only, was open to question

until recently, when Christie and Meakins (58) and Kaltreider and McCann (177) demonstrated impairment of distensibility and, to a lesser degree, of elasticity in vivo in cardiac decompensation. These abnormal findings tended to disappear with return of compensation.

It has been demonstrated in experiments in animals that changes in respiratory dynamics can occur reflexly as a result of pulmonary engorgement and increased pulmonary rigidity. The importance of these mechanisms in causing the paroxysmal dyspnea of cardiac asthma has been pointed out by Weiss and Robb (347). It is not unlikely that some of the constant dyspnea or the dyspnea of exertion in patients with chronic congestive failure is also a result of reflexes from the pulmonary vessels and parenchyma although no data are available.

V. ARTERIAL AND VENOUS BLOOD OXYGEN. ARTERIOVENOUS OXYGEN DIFFERENCE. TISSUE OXYGEN TENSION

The strikingly low oxygen content of the venous blood of patients with cardiac decompensation was first recorded by Means and Newburgh (225) in 1915 and subsequently by others (18) (54) (72) (76) (126) (152) (206) (207) (208) (274) (324) (346).

Diminution in the oxygen content and saturation of the arterial blood is a frequent finding in such patients (15) (16) (50) (54) (64) (76) (103) (126) (147) (152) (167) (173) (187) (274) (324) (346), though observed less regularly and to a lesser degree than that of the venous blood in heart failure.

An increased difference between the arterial and venous oxygen contents is the rule in cardiac decompensation whether measured directly by means of blood analyses or calculated from data obtained in estimating the cardiac output indirectly (see above). The increased arteriovenous oxygen difference found in patients with congestive failure is due to the decrease in the cardiac output discussed previously, as shown by the relationship:

$$\text{cardiac output} = \frac{\text{oxygen consumption}}{\text{arteriovenous difference}}$$

The low arterial blood oxygen saturation frequently observed in heart failure is due to a variety of causes, among which are inadequate

mixing of the air in the lungs (260) (311), organic changes in the alveolar walls, such as those described by Moshcowitz (238) and Parker and Weiss (246), and tachypnea itself. In regard to the last, Meakins and Davies (222) found that rapid shallow breathing reduced the arterial blood oxygen saturation in a normal subject from 94.3 to 91.7 per cent.

When a diminished arterial oxygen saturation occurs in uncomplicated heart failure, the values usually range between eighty-eight per cent and the lower limit of normal, ninety-four per cent; values below eighty-eight per cent are rarely found. Such decreases in oxygen saturation, though apparently small, are of great importance. To cause a reduction in his own arterial saturation to 88 per cent Barcroft (19) had to breathe air containing oxygen at a tension of below 100 mm. of mercury for two days. With his oxygen saturation at the above mentioned level, he became extremely ill, with severe dyspnea, headache, and vomiting. That apparently small changes in arterial blood oxygen saturation result in large changes in tissue oxygen tension is well known. Henderson (154) states that raising the blood oxygen saturation from 85 to 90 raises the tissue oxygen tension from 51.4 to 61.2, and increasing the blood oxygen saturation from 90 to 95 raises the tissue oxygen tension from 61.2 to 75.0 mm. of mercury.

No extensive studies of tissue oxygen tension in congestive failure exist. It is, however, apparent that since the tissue oxygen tension cannot be greater than that of the venous blood, the former must be lower than normal in patients with cardiac decompensation. Meyer's (230) data obtained by measuring the tissue oxygen tension directly in a few patients support this concept. The known causes of the abnormally low tissue oxygen tension are the slowing of the blood flow, the diminished arterial blood oxygen saturation, and the increased metabolic rate found in heart failure.

Additional evidence as to the existence of tissue anoxemia is offered by observations on the effects of oxygen therapy in congestive failure. Relief of dyspnea resulting from the administration of air enriched with oxygen was noted by Beddard and Pembrey (25), Barach and his co-workers (15) (16) (280), Campbell and Poulton (51), and Katz and Hamburger (64) (136) (178). Barach and Richards (15) (280) also

reported loss of edema in some cardiac patients as a result of oxygen therapy. Barach and his co-workers (15) (16) (280) found that relief of symptoms as a result of oxygen therapy was most likely to occur in patients in whom the arterial blood oxygen saturation was low; and was associated with a return to or toward normal values for arterial oxygen saturation.

VI. ALVEOLAR AIR AND ARTERIAL AND VENOUS BLOOD CARBON DIOXIDE.
CARBON DIOXIDE DISSOCIATION CURVES. ARTERIAL
AND VENOUS BLOOD pH

A lowering of the alveolar air carbon dioxide content or tension has been noted in patients with cardiac decompensation by many authors (25) (58) (62) (100) (107) (187) (201) (223) (251) (255) (258) (259) (261) (269) (282). The decrease in alveolar air carbon dioxide content and tension parallels the increase in respiratory minute volume and thereby is related to the degree of dyspnea to some extent.

Since the free carbonic acid of the arterial blood varies as the alveolar air carbon dioxide tension, the former must also be diminished in cardiac decompensation. A large number of observers have recorded measurements of the arterial whole blood total carbon dioxide content or tension, and the arterial plasma bicarbonate content in patients with congestive failure. In the great majority of patients with uncomplicated congestive failure the arterial blood total carbon dioxide content and tension are normal or low (15) (50) (54) (62) (64) (71) (76) (103) (105) (147) (152) (167) (173) (223) (261) (267) (280). The arterial whole blood carbon dioxide content and tension are elevated above normal levels in only a small minority of patients with cardiac decompensation. Such patients usually have organic pulmonary disease in addition to cardiac decompensation or else extremely severe pulmonary impairment due to engorgement resulting in interference with diffusion of carbon dioxide across the alveolar walls. The arterial serum bicarbonate is also usually normal or low, according to Scott (304), Peters, Bulger and Eisenman (262), and Richards and Barach (280), although some patients may show values somewhat above the upper limit of normal (262) (263). These latter patients usually have marked impairment of pulmonary func-

tion due to primary pulmonary disease, such as emphysema, or extensive fibrotic changes due to severe pulmonary congestion of long duration.

Since the pH of the blood depends on the ratio of bicarbonate to carbonic acid, it is clear that a variety of changes can occur in the arterial blood of patients with cardiac decompensation (15) (50) (62) (64) (71) (103) (105) (106) (187) (223) (261) (280). The arterial blood of most patients at rest yields values for pH within normal limits. An important group of patients with severe dyspnea occasionally show a tendency toward slight alkalosis; in such patients there is a marked lowering of the alveolar carbon dioxide tension with a consequent fall in carbonic acid content of the arterial blood. The ratio of bicarbonate to carbonic acid becomes increased and the arterial pH tends to rise. That more patients with cardiac dyspnea do not manifest this trend toward alkalosis is due to the increase in blood lactic acid (see below) so frequently present. A small group of patients, consisting principally of those with organic pulmonary disease, show carbon dioxide retention, with a consequent lowering of pH due to this factor in itself.

It is clear that changes in arterial carbon dioxide tension are usually the effect rather than the cause of dyspnea in patients with uncomplicated congestive failure at rest. If any change in the direction of increased acidity of the arterial blood occurs after exercise, as has been noted in some cases (71), it must be due to an increase in lactic acid (see below).

Changes in the venous whole blood carbon dioxide content and tension and serum bicarbonate at rest are also variable (54) (71) (76) (139) (152) (173) (255) (258) (267) (304), although lowering of the carbon dioxide content and tension such as occurs in the arterial blood is much less common. Consequently deviations from the normal venous blood pH due to changes in carbon dioxide tension are slight (71) (106) (139) (223) (248) (261) (267) (314). However, the difference between the resting arterial and venous blood pH is greater than normal, according to Meakins, Dautrebande, and Fetter (223) and Fraser, Ross and Dreyer (106). After exercise, Pilcher, Clark and Harrison (267), and Harris, Jones and Aldred (139) noted an abnormal lowering of the venous blood pH. The latter group of

investigators associated this increase in acidity with a rise in lactic acid content of the venous blood (see below). Simultaneously, (71) the venous blood carbon dioxide tension increases. It must be remembered, however, that general conclusions based on determinations on venous blood drawn from one extremity are to be accepted with caution.

The carbon dioxide carrying power of the arterial (15) (103) (105) (187) and of the venous (50) (139) (261) (314) blood not infrequently is diminished in patients with congestive failure, as manifested by lowering of the carbon dioxide dissociation curve. Harris, Jones and Aldred (139) associated the depression of the venous blood dissociation curve with an increase in lactic acid. Pearce (255) found a normal curve in one case.

Meakins, Dautrebande, and Fetter (223) determined the carbon dioxide dissociation curves in both arterial and venous blood in the same decompensated patients and found normal values for the former but a lowering in the latter. They ascribed this depression of the venous curve to loss of base to the tissues due to stasis (74). It is difficult to understand, however, how this lost base returns to the arterial blood so as to restore the curve of that blood to normal. Their findings are also difficult to interpret in the light of other workers who found a depression of the arterial carbon dioxide dissociation curve. Fraser, Graham and Hilton (104) corroborated the finding of Meakins, Dautrebande, and Fetter (223) as to the lowering of the venous curve as compared to the arterial, but in their experience this change was not limited to patients in whom stasis existed. They pointed out that the arterial blood carbon dioxide curve and pH were the result of the passage of the *mixed* venous blood through the lungs and that comparing arterial with venous blood taken from one part of the body, i.e., the arm usually, did not permit one to draw conclusions concerning the relation between the arterial and the *mixed* venous blood.

Whatever the cause of the lowering of the arterial and venous blood carbon dioxide curves in cardiac decompensation, the curves tend to return to normal with recovery.

After exercise the carbon dioxide combining power of the blood is markedly reduced in cardiac patients, according to Groag and

Schwartz (128) and Harris, Jones and Aldred (139). This shift toward acidosis is due to the abnormal accumulation of lactic acid in the blood (see below).

Peabody (249) in 1915 noted the abnormally increased sensitivity to carbon dioxide of patients with cardiac decompensation. Engelhard (84), Peters and Barr (259), and Pilcher, Clark, and Harrison (267) corroborated his finding of abnormally great respiratory responses to the inhalation of increased tensions of carbon dioxide. This phenomenon is probably related in part at least to the above described changes in the carbon dioxide carrying power of the blood, for the last named authors noted an abnormal lowering of the blood pH after such inhalations in cardiac patients, although retention of carbon dioxide was also increased. Abnormally increased sensitivity to carbon dioxide such as is known to occur in anoxemia may also be a contributory factor.

Patients in whom acidosis occurs might also be expected to show changes in the oxygen dissociation curve. Data bearing on this point are few. Lewis et al. (201) noted changes in the oxygen dissociation curve indicative of acidosis in the venous blood of some patients; Meakins, Dautrebande, and Fetter (223) obtained normal curves on the arterial blood in their cases of congestive failure.

Deviations from the normal in the arterial or venous serum bicarbonate content are associated with changes in the serum chloride level. Peters, Bulger and Eisenmann (262) (263) studied this problem and found that their cardiac patients could be divided into three groups: (1) patients with low bicarbonate and high chloride levels, (2) patients with high bicarbonate and low chloride levels (these patients usually have some associated pulmonary disease), (3) patients with low bicarbonate and chloride levels. The third group is a small one and represents those patients in whom there is probably an element of renal insufficiency accounting for the loss of serum base. The patients studied by Gilligan, Volk, and Blumgart (119) may be divided into similar groups. Richards and Barach (280) observed that a rise in serum bicarbonate content during recovery from congestive failure was followed by a fall in serum chloride level and a transient increase in the urinary excretion of chloride.

The changes in blood carbon dioxide and pH are as far as can be

ascertained, more the effect than the cause of increased respiratory activity. This does not, however, rule out acidosis of the respiratory center, since the reaction of the latter may become more acid than normal in spite of changes in the direction of alkalinity in the arterial blood delivered to the center.

VII. BLOOD LACTIC ACID AND OXYGEN DEBT

In 1913 Lewis, Ryffel, Wolf, Cotton, and Barcroft (201) reported an increase in the blood lactic acid content in some patients with congestive failure. Clausen (61) several years later noted a parallelism between blood lactic acid level and clinical condition in a decompensated patient. Many other observers have noted an increase in the resting blood lactic acid level in heart failure (1) (15) (24) (78) (139) (79) (172) (174) (224) (280) (300) (336) (346) and a general correspondence between the blood lactic acid content and the degree of cardiac decompensation. Weiss and Ellis (346), however, found no very striking changes in any of their cases. Abnormally large and prolonged rise in blood lactic acid occurs after exercise in patients with cardiac decompensation (78) (79) (139) (153) (174) (224). Groag and Schwartz (128) and Harris, Jones and Aldred (139) noted a simultaneous fall in alkali reserve. Groag and Schwartz (128), and Jervell (174) also found a very marked increase in lactic acid output in the urine after exercise in such patients; the severity of the clinical condition was related in a general way to the degree of lactic acidosis of the blood and urine. Weiss and Ellis (346) reported somewhat smaller rises in arterial and venous lactic acid content after exercise in their cases. Perger (256), Schumacher (300) and Beckmann (24) noted a slow rate of disappearance of injected sodium lactate in heart failure; according to Perger (256), the rate of oxidation varies inversely with the degree of failure.

Beckmann (24), Schumacher (300), Adler and Lange (1) and Valentin (336) felt that the disturbed lactic acid metabolism of cardiac decompensation was due to liver damage alone, since similar changes occur in primary liver disease. Dresel and Himmelweit (78) (79) found that the resting blood lactic acid level, as well as that after exercise on a staircase, might be similar in patients with heart and with liver diseases, but showed conclusively by means of dynamometer

experiments that metabolism of lactic acid of the peripheral tissues is defective in congestive failure but not in liver disease.

The relation of blood lactic acid accumulation to oxygen debt after exercise in normal individuals is well established and important. The oxygen debt of cardiac patients is abnormally large and prolonged (52) (85) (148) (156) (224) (242) (264). Most cardiac patients doing light work take up normal or slightly reduced amounts of oxygen during the performance of the work itself; those who perform work to the point of exhaustion absorb distinctly subnormal amounts of oxygen. The size and the duration of the oxygen debt after work varies in general with the deficit in oxygen intake during work. The elevated *resting* blood lactic acid in patients with cardiac decompensation can sometimes be abolished by inhalation of high concentrations of oxygen (15) (174) (280). Hewlett, Barnett, and Lewis (158) caused a reduction of as much as 75 per cent in the oxygen debt of normal subjects by allowing them to breathe air enriched with oxygen while exercising. That some patients are in oxygen debt even when at rest is also suggested by the findings of Jervell (174), Uhlenbruck (334), and Jansen, Knipping and Stromberger (173), who found that patients with cardiac decompensation took up abnormally large amounts of oxygen when exposed to high oxygen concentrations at rest. This "storage," according to Uhlenbruck (334) usually lasted for about forty minutes, following which the oxygen intake became normal. The markedly beneficial response to oxygen therapy in some patients with congestive failure is confirmatory evidence of the existence of a state of continuous oxygen debt.

It is apparent from all these observations that many patients with severe congestive failure develop abnormally great oxygen debts after exercise and may be in a state of oxygen debt even at rest. An obvious cause of this debt is delivery of insufficient amounts of oxygen to the tissues due to the decreased cardiac output and low arterial blood oxygen saturation of cardiac decompensation. The increased basal metabolic rate found in congestive failure is also a factor. Jervell (174) showed that anoxemia due to exposure to low oxygen tensions resulted in an increase in blood lactic acid content. The same result was, however, noted after venous stasis as well. That pulmonary factors by themselves may result in anoxemia and an

abnormally large oxygen debt was shown by Jacobeus, Nylin, and Almberg (171), who found abnormally large oxygen debts in normal individuals in whom the vital capacity was reduced by means of a tight chest binder. Since most patients with congestive failure have marked impairment of pulmonary function it is apparent that this in itself may be one of the causes for the abnormal debt of such patients. In general, therefore, the abnormal oxygen debt of patients with congestive failure is a result of tissue anoxemia presumably due to (1) low cardiac output together with peripheral stasis, (2) insufficient oxygenation of the blood in the lungs, and (3) increased metabolic rate.

VIII. THE VENOUS PRESSURE

Venous engorgement is one of the cardinal signs of congestive failure. Measurements of the venous pressure by many authors by direct or indirect methods have corroborated this clinical finding (15) (34) (35) (39) (60) (92) (93) (102) (114) (164) (168) (200) (213) (226) (237) (280) (299) (308) (340) (341). Hooker and Eyster (164), Eyster and Middleton (92) (93), Boas and Dooneief (39), V. Tabora (341) (237) and Meldolesi (226) reported normal venous pressures in patients with organic heart disease but without signs or symptoms of failure. On the other hand, Gaertner (114), Sewall (308) and Clark (60) found the venous pressure elevated in such patients. Schott (299), Blumgart and Weiss (34) (35), Harris, Jones and Aldred (139), Kinsman and Moore (184) and the author (4) found much overlapping of venous pressure values when normal individuals, uncompensated cardiac patients, and patients in failure were compared.

The rise in venous pressure frequently found in cardiac decompensation is due mainly to the inability of the heart to take up and propel forward all the blood brought to it. This concept is supported by the fact that the rise of venous pressure which follows exercise is greater and more prolonged in cardiac patients than in normal subjects (145) (299). The normal response to an increase in venous pressure is an increase in cardiac output (7); decompensated cardiac patients who show increased venous pressure at rest have diminished cardiac outputs. Hyperpnea is another contributory factor in increasing venous pressure, since increased respiratory activity raises the intrapleural

pressure (272) and impedes the entrance of blood into the thorax (163). Pulmonary congestion has a similar effect since it raises the intrapleural pressure (58).

When the venous pressure is elevated in cardiac decompensation it usually falls to or toward normal during recovery. Of all therapeutic procedures venesection is the most rapidly effective in lowering the venous pressure (60) (93) (200) (283) (333) (341). The bearing of this finding on the relief of orthopnea will be discussed in another place. Failure to fall, or a continuous rise of venous pressure may be a poor prognostic sign. Not infrequently an elevated venous pressure is found weeks or months before dyspnea, orthopnea, and edema appear. In many instances, however, marked signs of failure, such as râles in the chest, cyanosis, hepatic engorgement or even edema, may be present without any elevation in venous pressure.

IX. CEREBROSPINAL FLUID PRESSURE

The relation between venous and spinal fluid pressures in normal subjects is well established. Lamache (192) in 1926 first noted an increased spinal fluid pressure in cardiac decompensation. This observation has been amply confirmed (108) (150) (151) (228) (270) (283) (333). Harrison (150) also showed that the cisternal pressure is elevated. The spinal fluid pressure parallels the venous pressure and is always somewhat the higher of the two. With recovery from congestive failure the two measurements fall to or toward normal levels, though the actual or percentage decrease in both is not necessarily the same. Patients with considerably elevated spinal fluid pressure due to cardiac decompensation show none of the symptoms or signs usually associated with similarly increased intracranial pressure, due to primary intracranial disease such as headache, coma, vomiting, slow pulse, or papilledema. This fact suggests that factors in addition to the cerebrospinal fluid pressure itself may be important in the genesis of these signs and symptoms in primary intracranial disorders.

Tzanck and Renault (333) and Robertson and Fetter (283) demonstrated the effect of venesection in lowering the spinal fluid pressure as well as the venous pressure. The latter authors noted the disappearance of orthopnea following this procedure; they believed more

striking relief followed simultaneous withdrawal of spinal fluid. Harrison (151) observed improvement in orthopnea in most instances after spinal drainage; the peripheral venous pressure also usually decreased significantly after this procedure. Harrison (151), however, was unable to correlate the relief of orthopnea with lowering of the venous pressure in his cases, and felt that orthopnea was due to increased intracranial pressure. Patients with increased intracranial pressure due to local cerebral causes do not have orthopnea, however, nor does lumbar puncture alter their respiratory dynamics.

It must be concluded that a relation between increased intracranial pressure and orthopnea in heart failure is not demonstrated. The reason for the decrease in peripheral venous pressure following spinal puncture is also obscure.

X. PLASMA AND EDEMA FLUID PROTEINS

A low serum protein level in several patients with cardiac decompensation was described by Hoffman (161) in 1883. Since that time his observation has been amply confirmed by numerous investigators (12) (20) (23) (44) (68) (83) (101) (118) (119) (125) (169) (170) (191) (204) (239) (247) (280) (287) (294). As pointed out by Payne and Peters (247) and Thomson (330) the serum albumin is more likely to be affected than the serum globulin. Moore and Stewart (236) found a normal serum protein level in patients without edema. Barath and Elias (17), Meyer (231), Thomson (330), Mayrs (216), Horsters (165) and Smirk (312) noted a lowered plasma oncotic pressure in congestive failure. In a general way, low protein levels or diminished plasma osmotic pressures have been associated with the presence of edema but in large series of cases, such as those of Payne and Peters (247) and Cope (68), a high degree of correlation between the presence of edema and a decrease in serum protein level does not exist. Smirk (312) showed that decreases similar to those observed in patients with edema due to congestive failure are not associated with the presence of edema in non-cardiac patients.

Payne and Peters (247) regarded malnutrition as the principal cause of the diminution in serum protein content but other authors, including Iversen and Johansen (169) and Ehrstrom (83) regard the marked albuminuria frequently seen in severe cardiac decompensation as an

important contributory cause. Loss of protein through repeated paracentesis may also be a factor in depressing the serum protein level, for relatively large amounts of protein have been found in chest fluids (118) (119) (161) (170) (294) (337) and in abdominal fluids (118) (119) (161) (204) (278) (294) (337) in congestive failure. The development of edema itself is associated with a fall in serum protein level, according to Atchley, Loeb, Benedict and Palmer (12) and Iversen and Johansen (170). The serum protein content increases after the disappearance of edema (12) (20) (119) (280) (170). The decrease in serum protein level associated with edema formation may be due to loss of some protein into the interstitial tissues. That some protein does pass into tissue fluid when the venous pressure is elevated has been shown by Senator (306) and Landis et al. (196). Another cause for the lowering of the plasma protein level in congestive failure may be dilution of the blood plasma consequent to the increase in blood volume which occurs in this condition.

Studies of the protein content of subcutaneous fluid in congestive failure have been recorded by many investigators (23) (44) (94) (101) (118) (119) (125) (162) (191) (294) (306) (337). The reported values have usually been less than 0.6 gm. per cent although occasional values of 1.0 gm. per cent or more have been found. Beckman (23), Fodor and Fischer (101), and Gollwitzer-Meier (125) found the edema fluid of cardiac patients two to seven times as rich in protein as that of nephrotic patients. Bramkamp (44) detected little or no globulin in subcutaneous edema fluid drawn from patients with cardiac decompensation. Petersen and Willis (265) have advanced an attractive hypothesis which uses the ratio between the serum protein and that of the exudate or transudate as a measure of permeability of the vessel involved. According to their formula the permeability of the tissue capillaries is much increased in congestive failure. However, their hypothesis has no experimental basis.

Senator (306) in 1888 recorded the interesting observation that putting a tourniquet around the limb of a patient with cardiac edema may raise the protein content of the edema fluid by as much as forty per cent. More recently Landis, Jonas, Angevine and Erb (196) have corroborated this finding, noting an edema fluid protein level, of 1.8 gm. per cent at very high venous pressure artificially produced in

normal subjects, as compared with 0.3 gm. per cent at lower venous pressures.

XI. BLOOD VOLUME AND VISCOSITY. HEMOGLOBIN AND ERYTHROCYTE COUNT

Most authors report an increased blood volume in patients with congestive failure (46) (116) (160) (181) (268) (302) (331). Bock (40) described normal values in his one case. Wollheim (349) and Goldbloom and Libin (123) found either an increased or a decreased blood volume in patients with cardiac failure and evolved the theory of two forms of failure, i.e., "plus" and "minus" to explain their findings. The dye method used by these authors has been severely criticized (127), however, so that the validity of their theory is questionable.

After recovery from congestive failure, following digitalis or other forms of therapy, a decrease in blood volume occurs, according to Bock (40), Thompson (331), Schurmeyer (302), Hitzengerger and Tuchfeld (160), Mies (232), Ewig and Hinsberg (91), and Gibson and Evans (116), while Wollheim (349), Goldbloom and Libin (123), Brown and Rowntree (46), and Ehrstrom (82) noted either an increase or a decrease. The most complete studies, and those most free from errors inherent in technique are those recently reported by Gibson and Evans (116). These authors found a high degree of correlation between the severity of signs and symptoms of congestive failure and increase in blood volume, corroborating and extending Thompson's (331) earlier findings during the course of failure in one patient. In recovery from congestive failure the blood volume usually decreases toward, but rarely attains normal levels. The reason for the changes in blood volume in congestive failure cannot be stated. Judging by changes in plasma protein (see above), it would appear that some dilution of the blood plasma occurs in patients with edema due to heart disease, but the cause of this phenomenon is also unknown. Gibson and Evans (116) and Brandt (45) found a parallelism between changes in blood volume and venous pressure in individual cases; but the increased blood volume was not responsible for any more than a small part of the venous pressure rise, since diuresis caused marked decreases in blood volume and only small changes in venous pressure. The general vasodilation suggested by increased number of capillaries

visible in the skin of decompensated cardiac patients is probably also related to the abnormally large blood volume. Whether generalized capillary dilatation results in increased volume or vice versa cannot be stated with certainty, however.

It is possible that the increased blood volume of cardiac decompensation represents an attempt to compensate for a low cardiac output. It is obvious that if the flow of blood through a unit volume of tissue is low, anoxemia will result. This can, to some extent, be obviated if a larger volume of blood is kept in contact with the unit volume of tissue for a longer period of time. It is interesting in this connection that the signs and symptoms of surgical shock may be induced in patients who enter the hospital with severe congestive failure if they are dehydrated, with resultant marked decrease in blood volume, too rapidly, i.e., in a period of three or four days, by too enthusiastic diuretic therapy.

The red blood cell count and hemoglobin percentage show no consistent change in cardiac decompensation. In most cases, normal values are observed. Patients in severe congestive failure, especially those with marked cyanosis, may show an elevation of the red blood cell count and hemoglobin percentage. However, in view of the increase in total blood volume in all patients in failure, it must be concluded that the *total* red blood cell count is always increased in cardiac decompensation. In many instances a tendency toward increased corpuscular volume occurs.

Ehrström (82) studied the changes in the blood during the various phases of congestive failure and found reticulocytosis with the onset of decompensation and evidences of blood destruction during recovery. These findings point to anoxemia as the cause of the increased total red blood cell count of congestive failure. The fragility of the red blood corpuscles is increased in patients with cyanosis due to heart disease (126a).

Changes in blood viscosity might be expected to occur when the number of red blood cells per cubic millimeter is increased. Bence (26) and Markson (214) observed such increased viscosity of the blood in some patients with cardiac decompensation; they stressed both the increase in number and in mean cell volume of the erythrocytes as causes. Bence (26) showed that administration of oxygen to such patients caused a return to normal of the viscosity of the blood.

XII. PERIPHERAL CAPILLARIES

Engorgement of the tissue capillaries, often to the point of hemorrhage, is the usual post mortem finding in congestive failure. The degree of congestion which exists during life in such patients cannot be estimated accurately from sections of fixed tissues, since some of the changes are unquestionably agonal or post mortem. Direct observation of the capillaries of the nail folds of patients with congestive failure has been possible for many years. The findings of all observers (69) (70) (109) (159) (176) (241) (285) (301) (305) (343) are in substantial agreement. The visible capillaries are increased in number, show dilatation of the venous loop, and may exhibit narrowing of the arterial loop. The blood in them is dark, moves slowly, and may be motionless for abnormally long periods of time. The column of moving blood appears "granular" or "segmented." It is to be noted also that the small venules of the subpapillary plexus are likewise abnormally numerous and prominent. Hisinger-Jagerskiöld (159) found these changes only in patients with peripheral failure and not in those in whom the signs of congestion were limited to the lungs. The reason for the dilatation of the venous limb of the peripheral capillaries is made clear by the observations of Krogh (189), who showed that anoxemia causes capillary vasodilatation. In congestive failure the effects of anoxemia are naturally most marked at the venous end of the capillaries.

Unfortunately no direct measurements of the capillary pressure in patients with congestive failure are available though it is obvious that the capillary pressure must be higher than the venous pressure unless reversal of flow is to occur.

XIII. LYMPHATICS

The recent studies of McMaster (219) on the lymphatics of the skin in patients with congestive failure throw additional light on the processes involved in the formation of cardiac edema. This author found dilatation and valvular incompetence of the lymphatic channels and complete absence of lymph flow in edematous cardiac patients. The administration of diuretics caused no change in the picture. Elevating the edematous limb, however, caused small increases in lymphatic flow. McMaster's inability to reproduce the typical

lymphatic stasis of congestive failure by means of tourniquets in normal subjects caused him to rule out increased venous pressure as the cause of the lymphatic stasis in cardiac decompensation observed by him; anoxemia may be the factor responsible. It is probable, however, that the elevated venous pressure, found in many patients with congestive failure, is also a factor preventing normal lymphatic function, for increases in venous pressure must be transmitted to at least the larger lymphatic vessels.

XIV. TEMPERATURE REGULATION. INSENSIBLE PERSPIRATION

Slight to moderate elevation of body temperature in the absence of evidences of infection is frequently observed in patients with congestive failure. Cohn and Steele (63) studied this phenomenon and found a close parallelism between the presence of signs of congestive failure and elevation of temperature, except that the latter might occur before the former became evident. Steele (321) further reported that the difference between the skin and rectal temperatures is abnormally great in cardiac patients and that such patients eliminate approximately three times as much heat as normally via the lungs (322).

These findings are not unexpected, in view of the known marked reduction in cardiac output. Such diminution in blood flow to the periphery necessarily affects the dispersal of heat generated within the body, thereby giving rise to an elevation of body temperature. Steele (323) was able to reproduce these conditions in normal subjects by slowing the peripheral circulation by means of tourniquets. He suggested (321) that this rise in body temperature might be one cause of the hyperpnea exhibited by patients with cardiac decompensation. This concept is supported by the clinical observation that many patients with severe congestive failure claim to experience increase of dyspnea when wrapped in warm blankets and placed in a warm, stuffy room.

Additional evidence as to the existence of impaired heat dispersal through the skin is afforded by the observations of Zak (350), Conti (67) and Kesterman and Schleining (180) who found that the insensible perspiration is greatly diminished in congestive failure.

XV. BASAL METABOLIC RATE

Elevation of the basal metabolic rate in congestive failure has been noted by many authors (76) (86) (135) (152) (156) (184) (223) (250) (254) (324) (345).

Peabody, Wentworth and Barker (254) and Resnik and Friedman (276) found a high degree of correlation between the degree of dyspnea and the rise in metabolism. The latter authors felt that the muscular effort associated with dyspnea was the cause of the change in metabolism. However, the slight temperature rise found in severely decompensated patients may also be a factor. Both elevation of temperature and severe dyspnea occur in the most severely decompensated patients, and the increased metabolism could very well be related to both at the same time.

It is of interest that the elevated basal metabolic rate of congestive failure is not associated with the abnormally high insensible perspiration which occurs in thyrotoxicosis; the insensible perspiration in congestive failure is usually diminished.

The degree of elevation of the basal metabolic rate found in some cases may be misleading in that the values obtained may be too high. This is due largely to the fact that it is frequently impossible for the sick uncomfortable cardiac patient to assume a truly basal state. Another factor leading to error in measuring basal metabolism in severely decompensated patients is the fact that such patients, when exposed to the high concentration of oxygen in the ordinary type of basal metabolism apparatus take up excessive amounts of oxygen in an attempt to discharge their oxygen debt.

XVI. LIVER FUNCTION

Hepatic enlargement is one of the most frequent and often one of the earliest signs of cardiac decompensation. It may be noted weeks or months before edema or rales can be detected. This phenomenon is readily understandable in the light of Brunton's (48) observations on the enormous distensibility of the liver. In very severe failure jaundice may appear, with bile in the urine, but as a rule this is not the case. In spite of the absence of frank jaundice, however, some increase in the serum bilirubin is common in cardiac decompensation

(1) (10) (21) (27) (96) (99) (117) (120) (175) (229) (245) (266) (275) (295) (297) (339) (342). In general, the degree of elevation of the serum bilirubin corresponds with the severity of the cardiac failure, but values above 2.0 mg. per cent are not common. However, the degree of bilirubinemia is not necessarily related to the increase in size of the liver (96) (99) (175) (295). Liver function tests are less likely to show abnormalities than estimation of the serum bilirubin in congestive failure. The rose bengal test is normal in cardiac decompensation (88) (175) (245) (307) and Joliffe (175) also found the levulose tolerance test of little value in this condition. A normal galactose tolerance was found by Adler and Lange (1). Meakins (220) found that jaundice, when it occurred, was most marked over the upper chest and face. He also stated that bile pigment is not found in edema fluids. Andrews (10), however, detected appreciable amounts of bilirubin in ascitic fluid taken from a patient with cardiac failure and the author (8) has also found it in this as well as other edema fluids in cardiac patients with normal or elevated serum bilirubin levels. The quantity of bilirubin present in edema fluids roughly parallels but is always less than that found in the serum at the time; the protein content of the edema fluids also influences, to some degree, the bilirubin content (8).

The abnormal liver function found in cardiac decompensation is due to the typical histological changes found in the liver (112) (193) (212) (220) (244). Meakins (220) related the degree of hepatic engorgement and central necrosis to the increased venous pressure of cardiac decompensation. Bolton (43) and Zimmerman and Hillsman (351) were able to reproduce the typical pathology by obstructing the inferior vena cava in animals. Mallory (212), however, pointed out that if back pressure were the cause of the necrosis, it should involve the entire liver lobule, since the increased pressure must be distributed everywhere. Mallory felt that a toxin of some sort was responsible. Indeed the pressure actually is higher in the periphery of the liver lobule if the gradient of pressure necessary for flow of blood is to be maintained.

It is probable that anoxemia is the important factor in the genesis of the altered histology and physiology of the liver in cardiac decompensation. Neubauer (240) and Mattson (215) showed that anoxemia

caused a striking increase in size of the liver in animals. Rich and Bumstead (279) found the typical histological changes of congestive failure in the livers of patients with anoxemia due to severe anemia and in animals exposed to low oxygen tensions. Resnik and Keefer (277) observed central liver necrosis in anoxemic dogs. These authors, as well as Barron and Rich (22) observed a low liver function as measured by the bilirubin excretion test in such animals.

Available evidence indicates that the hyperbilirubinemia of cardiac decompensation is not due solely to faulty excretion of bile pigment, for several observers (82) (89) (96) (266) (342) have found increased amounts of urobilin in the stool or urine, or both. Eppinger (87) found an increased amount of bilirubin in the duodenum. These findings point to increased production of bile pigment in the body. Increased fragility of the red blood cells has been reported in patients with cyanosis due to heart disease (126a). Ernstene (89) pointed out that the jaundice of heart disease is due to a combination of factors, i.e., increased production and diminished excretion of bile pigment.

The diminished liver function in heart failure is in itself rarely severe enough to be clinically important. It is of significance in that it indicates the presence of liver damage which may lead to cirrhosis. That so few patients with heart failure develop clinically important cirrhosis of the liver is unquestionably due to the fact that very few of them live long enough. Zimmerman and Hillsman (351) were able to reproduce in animals the central scarring which is said to be typical of cardiac cirrhosis. Bolton (43) in his experiments observed no central fibrosis but did occasionally find some portal infiltration and scarring. This observation is especially significant in view of the fact that not infrequently patients in whom a clinical diagnosis of "cardiac" or central cirrhosis of the liver is made, are found, at post mortem, to show unmistakable evidences of Laennec's cirrhosis.

XVII. RENAL FUNCTION

Striking changes in renal physiology are of frequent occurrence in congestive failure although pathologically not much more than congestion and edema of the kidney is to be found.

Albuminuria is almost a constant finding in moderate or severe heart failure. Rowntree, Fitz, and Geraghty (289) were able to

cause the appearance of albumin in the urine by partially obstructing the renal veins in animals. Clinically, however, albuminuria occurs in many decompensated cardiac patients in whom the peripheral venous pressure is within normal limits. Albuminuria in congestive failure is usually of moderate degree. At times, however, it may approach in quantity that observed in severe renal disease.

Stewart and Moore (328) investigated the formed elements in the urine in heart failure and observed a rough parallelism between the number of casts and leucocytes and clinical condition in a group of patients; erythrocytes tended to persist in abnormal numbers even after recovery from cardiac failure. Rowntree, Fitz, and Geraghty (289) in their experiments on obstruction of the renal vein in animals showed that the number of formed elements in the urine corresponded to the degree of renal congestion.

Various investigators have studied the physiology of the kidneys in heart failure by means of renal function tests. Rowntree and Fitz (288) many years ago reported a high urinary specific gravity in cardiac decompensation. This has been correlated with the low urinary output usually observed in edematous patients. The blood non-protein nitrogen is usually normal in cardiac decompensation but in severe failure, nitrogen retention may occur (2) (95) (113) (288) (290) (327), the blood non-protein nitrogen concentration occasionally reaching sixty or seventy milligrams per cent.

The above authors have also reported moderately diminished phenolsulphonphthalein excretion in congestive failure, with restoration of this function to normal after recovery. Rowntree, Fitz and Geraghty (289) were able to reproduce these findings in animal experiments by obstructing the renal veins. Diminished blood flow, in itself, without the element of venous engorgement, must be regarded as an important factor in impairment of renal function for Van Slyke, Rhoads, Hiller and Alving (338) have demonstrated a close parallelism between urea clearance and the volume of blood flow through the kidneys in dogs.

In general, as pointed out by Stewart and MacIntosh (327), decreased renal function is most likely to occur in patients with cardiac failure who are over thirty years of age, especially those with decompensation due to arteriosclerosis or hypertension. Under any

circumstances, however, the impaired renal function of congestive failure is of no great importance. It is rarely very severe and it is always transient, disappearing with recovery from failure. This in itself should never be the sole reason for ruling out the use of mercurial diuretics.

XVIII. GASTRO-INTESTINAL FUNCTION

Anorexia, nausea, distension, gaseous eructations, and flatulence are frequent complaints in moderate or severe congestive failure. An explanation for these symptoms is offered by the finding of van Liere and his coworkers (70A) (337A) (337B) that gastric tonus is decreased, peristaltic and hunger contractions diminished, and emptying time prolonged in the presence of anoxemia. No diminution in gastric secretory activity was noted (311A) at levels of anoxemia found in cardiac decompensation. The observed impairment of gastro-intestinal function probably is responsible, at least in part, for the loss of weight which occurs in congestive failure.

XIX. CREATINE METABOLISM

Kindler (182) studied the incidence of creatinuria in patients with cardiac decompensation and found it present in all patients with signs of severe failure. The creatinuria disappeared with improvement in the cardiac decompensation. Kindler believed this deviation from the normal creatine metabolism was a result of the effect of anoxemia on muscle.

It is of interest that many patients with cardiac decompensation complain of generalized muscular weakness; in some instances it is more prominent a symptom than dyspnea. Such muscular weakness may be related to the abnormal creatine metabolism observed by Kindler in patients with heart failure.

PART TWO

The foregoing pages contain a summary of the data now available on the physiology and chemistry of cardiac decompensation. In the following section an attempt will be made to relate these data to the pathogenesis of the signs and symptoms of that disorder.

result in the appearance of edema as in malnutrition or the nephrotic syndrome. Such marked lowering is, however, rarely encountered in uncomplicated cardiac decompensation; lesser decreases in plasma protein content are the rule, i.e. to about 5.5 to 6.5 grams per hundred cc. Decreases of this magnitude are, however, of importance in the genesis of edema when combined with abnormalities in other factors.

The mechanisms resulting in decreased plasma protein concentration are many and include the effects of malnutrition, albuminuria, and the loss of body proteins as a result of abdominal or pleural paracenteses. The loss of protein to the tissues when edema fluid is forming and the dilution of the blood protein consequent to the increase in plasma volume which occurs in congestive failure are likewise significant.

3. *Increased Venous Pressure.* Elevation of the venous pressure is an important factor favoring the elaboration of increased amounts of tissue fluid. A series of observations including those of Mende (227), Drury and Jones (81) and Landis et al (190) (195) (196) have established this relationship on a quantitative basis. It is clear that extreme rises in venous pressure, i.e. to thirty or forty centimeters of water, may by themselves cause the appearance of edema. However, the relation of the moderate increases in venous pressure commonly found in congestive failure to edema formation is not constant. Clinically it has long been known that some patients with venous engorgement do not have edema and that, conversely, some patients with cardiac edema do not have venous engorgement. The observations of Schott (299), Blumgart and Weiss (34) (35), Kinsman and Moore (184), and Harris, Jones, and Aldred (139) showed much overlapping of venous pressure values in edematous and non-edematous cardiac patients. It was, therefore, decided to re-investigate this question by a study of a large series of patients (5). One hundred and sixty-six patients were studied. No patients with myxedema or with nephritis were included because of their known tendency toward the elaboration of extracellular fluid. Similarly patients with marked emphysema were excluded from this study because many of them have slight elevations of the venous pressure not due to cardiac decompensation.

For the purposes of analysis, the patients studied were divided into four groups, as follows:

Group I: 83 cases. Patients with no history or symptoms of congestive failure.

Group II: 33 cases. Patients with congestive failure who had never had peripheral edema.

Group III: 35 cases. Patients who had had edema due to congestive failure, but who were rendered edema-free by the usual therapeutic measures and did not regain edema while under observation in the hospital.

Group IV: 15 cases. Patients who required an injection of a mercurial diuretic at regular intervals, usually a week or ten days, to dissipate the edema which was constantly forming while the patients were at rest in bed.

The venous pressure in each instance was measured by the direct method of Moritz and Tabora (237). The observations were made not less than three hours after the last meal and after a rest period of at least an hour.

The venous pressure was below +10 cm. of water in 79 of 83 normal subjects (Group I). In only three of these cases was it above +11 cm. of water. The venous pressure was above +10 cm. of water in seven of 33 (21%) decompensated cardiacs who had never had edema (Group II); the highest venous pressure in this group was +14.5 cm. Eleven of the 35 patients (31%) who were rendered edema-free and did not regain peripheral edema had venous pressures above +10 cm. (Group III); in this group it ranged as high as +13.9 cm. In only eight of the 15 patients who were forming edema fluid at rest in bed was the venous pressure greater than +10 cm. In the remaining cases of this group (Group IV) it ranged between +0.8 cm. and 7.1 cm.

The above observations on the lack of correlation between increase in venous pressure and the presence of edema have been corroborated by the studies of Altschule and Blumgart (6) in a patient with tricuspid stenosis. The venous pressure in this case as measured in both the antecubital and femoral veins ranged between 16 and 22 cm. of water, levels frequently associated in cardiac patients with the presence of edema. However, the patient studied was free of edema. It was felt that the absence of edema in spite of a high venous pressure was due to the fact that other factors which may be responsible for

edema, viz., low cardiac output, deficient oxygenation of the blood, and low plasma protein levels, were not present in this patient.

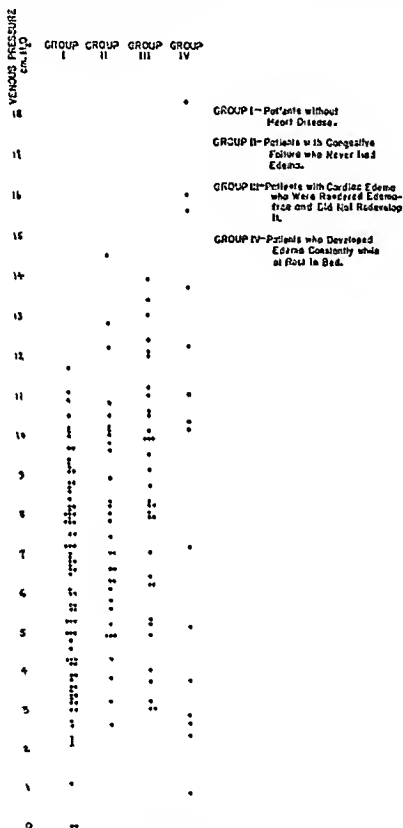


FIG. 1. VENOUS PRESSURE AND EDEMA FORMATION

More recently Smirk (312) in his above quoted experiments has shown that a rise in general venous pressure such as is commonly found in patients with cardiac decompensation is not in itself sufficient to cause the development of pitting edema.

It must be concluded that while extreme rises in venous pressure, i.e. to levels of thirty to forty centimeters of water, may by themselves cause the appearance of edema, the moderate increases in venous pressure commonly found in congestive failure constitute an important contributory factor but do not result in edema formation unless additional influences are operating at the same time. The venous pressure rise seen in cardiac decompensation is itself due to a multiplicity of factors, including the accumulation of blood in the veins due to a low cardiac output, increased intrapleural pressure due to hyperpnea and to congestion of the lungs and probably also the increase in blood volume which occurs in decompensated cardiac patients.

4. Abnormal Lymphatic Function. Clinical and experimental observations have conclusively proved the occurrence of edema following injury to lymphatics. It remained for McMaster (219) to demonstrate the absence of normal lymphatic flow in patients with cardiac edema. This is in marked contrast to patients with nephritic edema, in whom he found striking lymphatic hyperactivity. The mechanisms underlying the change found in congestive failure are obscure; it is possible that both a rise in venous pressure and anoxemia may be implicated. Whatever the causes of this phenomenon, however, it is apparent that this factor is of great importance in the genesis of cardiac edema.

5. Diminished Tissue Pressure. The elaboration of tissue fluid depends not only on intravascular conditions favoring transudation but also on extravascular factors which resist the escape of fluid into the tissues. Landis and his coworkers (190) (195) have recently demonstrated quantitatively the action of tissue pressure in inhibiting the accumulation of abnormal amounts of tissue fluid. The ease with which edema develops in cachectic patients with lax subcutaneous tissues exemplifies the importance of lowered tissue pressure in accelerating the formation of edema. Loss of flesh due to malnutrition is recognized as frequently present in chronic congestive failure. That repeated bouts of edema followed by loss of edema as a result of diuresis, results in a more or less permanent impairment of tissue elasticity has long been suspected. Unpublished observations made in this clinic demonstrate that patients who have had repeated periods

of cardiac decompensation harbor two to four times as much invisible edema as those in their first attack of congestive failure. In agreement with the foregoing considerations concerning the rôle of a reduced tissue tension in the genesis of edema are certain clinical observations on patients with adhesive pericarditis. It has been known for many years that these patients exhibit ascites early in their course, and edema of the legs late, if at all (*ascites praecox*). A reasonable explanation for this phenomenon is as follows: These patients have an elevated venous pressure which tends to result in edema. However, because of the fact that dyspnea is often negligible in this disease, the patients are up and about until late in their course. They, therefore, receive the benefit of the pumping action of the leg muscles and avoid the loss of tissue tension which follows prolonged bed rest. The only site where edema fluid can accumulate is within the abdomen, where muscular tension is low.

All of the foregoing considerations point to the conclusion that low tissue tension, due to malnutrition and also to the flaccidity of muscles which results from the necessarily prolonged bed rest, is a potent factor in the genesis of cardiac edema.

6. *Capillary Dilatation.* The widespread capillary dilatation in decompensated patients, with its resultant increase in filtering surface area enhances the tendency toward edema formation due to elevation of the venous pressure. In patients in whom the venous pressure is normal it is extremely doubtful whether an increase in filtering surface favors the development of edema.

7. *Sodium Chloride Intake.* Clinicians have long been aware of the relation of sodium chloride intake to edema formation in congestive failure. Indeed Coller, Dick, and Maddock (65) have recently demonstrated conclusively that excessive salt intake will result in the appearance of edema even in normal individuals. With many factors favoring the development of edema in cardiac patients, it is clear why merely normal amounts of ingested salt may precipitate the appearance of pitting edema in such individuals.

8. *Alkalosis.* Alkalosis clinically is frequently associated with edema formation, but it is extremely unlikely that the slight degree of alkalosis of the arterial blood that may occur in markedly dyspneic patients is of importance in the genesis of cardiac edema.

9. *Conclusions.* A general conclusion based on all the above considerations is that the edema of congestive failure is usually not due to the operation of any one factor. It is impossible to correlate with absolute agreement the presence of edema with changes in any one of the above discussed factors. Extreme changes in a single factor, such as the venous pressure or plasma protein level may in themselves result in the appearance of edema, but such extreme changes occur only uncommonly in uncomplicated cardiac decompensation. It appears that the formation of cardiac edema in most patients is the result of a combination of sub-maximal, or even minimal changes in

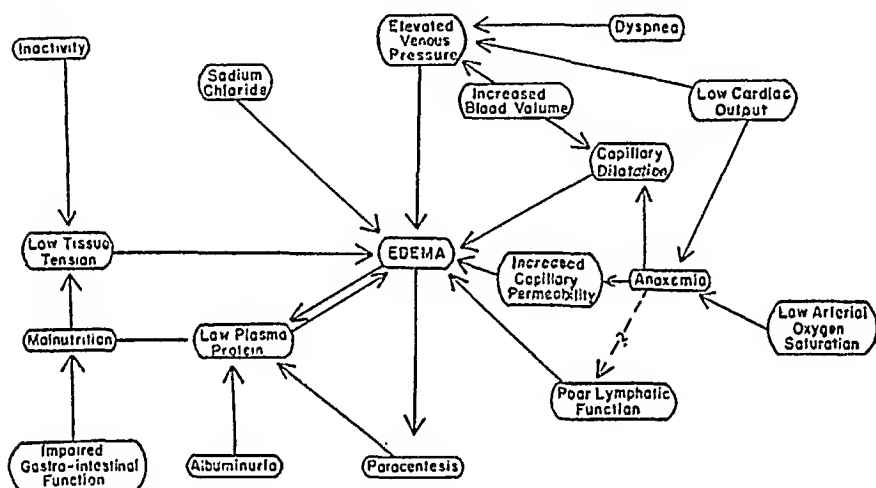


FIG. 2. THE MECHANISM OF EDEMA FORMATION IN CONGESTIVE HEART FAILURE

many factors. The relative importance of each of the above discussed factors unquestionably varies from patient to patient.

B. CYANOSIS

Cyanosis, or blueness of the skin, is a common finding in chronic cardiac decompensation. Its onset frequently precedes that of edema and orthopnea but rarely, except in patients with congenital heart disease, that of dyspnea. Its earliest manifestations consist of slight blueness in areas where the skin is thinnest, i.e., the nail-beds and lips. When generalized, it is usually most marked over the extremities of the body, i.e., fingers, toes, nose, ear-lobes and lips.

Lundsgaard and Van Slyke (209A) have defined the conditions under which cyanosis may occur. The factors of greatest importance in the cyanosis of congestive failure are (a) changes in the blood present in the skin and (b) the amount of blood in the skin. It has been demonstrated (209A) that the presence of unsaturated hemoglobin in a concentration of at least five grams per hundred cubic centimeters of blood is necessary for the production of cyanosis. Lundsgaard and Van Slyke (209A) pointed out that the low blood hemoglobin concentration of severe anemia may prevent the appearance of cyanosis; conversely, the presence of polycythemia facilitates the development of cyanosis.

A high concentration of reduced hemoglobin can result from (a) lowering of the arterial oxygen saturation before the blood reaches the tissues or (b) extraction of abnormally large amounts of oxygen from each unit volume of blood in the tissues.

1. *Decreased Arterial Blood Oxygen Saturation.* Decreased arterial blood oxygen saturation due to inadequate aeration of the blood in the lungs is frequently found in congestive failure. Its importance in the genesis of cyanosis of congestive failure is conclusively shown by the marked lessening of cyanosis which follows administration of air enriched with oxygen; accompanying this improvement is a return to or toward normal of the arterial blood oxygen saturation. However, relief of cyanosis due to cardiac decompensation resulting from oxygen therapy is frequently only partial; in addition, the degree of cyanosis cannot be exactly correlated with the arterial oxygen saturation. These facts suggest that other factors also operate.

2. *Increased De-Oxygenation of Capillary Blood.* Abnormally great de-oxygenation of the capillary blood occurs in congestive failure as a result of a lowered cardiac output and consequent slowing of the peripheral blood flow. Concentrations of reduced hemoglobin well above five grams per hundred cubic centimeters of blood may occur, with resultant appearance of cyanosis.

3. *Capillary and Venous Dilatation.* The widespread capillary dilatation and increased prominence of the subpapillary venous plexus exhibited by patients with cardiac decompensation exaggerates the degree of blueness present. Indeed, Goldschmidt and Light (124) have shown that these phenomena when induced in normal indi-

viduals may in themselves cause cyanosis even in the absence of abnormal blood gas values.

It is evident, therefore, that the cyanosis of congestive failure like edema is due to a multiplicity of factors. All factors operate in greater or lesser degrees in different patients, although the relative importance of each factor varies from patient to patient.

C. DYSPNEA

Before entering into a discussion of the pathogenesis of cardiac dyspnea it would be well to call attention to certain general considerations. It cannot be emphasized too often that dyspnea is a *sensation*, and as such is not amenable to objective measurement. There is a general correlation between the symptom dyspnea and the sign hyperpnea; the latter can be estimated by physical means. Marked differences in degrees of dyspnea due to changes in subjective sensitivity can, however, occur in different patients in whom all physiological measurements are the same, or even in the same patient with relative constancy of the physical status. These facts necessarily make it difficult to evaluate accurately the significance of the physiological and chemical changes which lead to dyspnea.

It must be remembered in addition, that hyperpnea is a response to certain abnormalities in the physiological or chemical status of the patient and that the hyperpnea may cause compensatory changes in the opposite direction, so that deviations from the normal observed, for instance in the blood, may be negligible.

For the purposes of this discussion no attempt will be made to distinguish dyspnea at rest from dyspnea on exertion. The assumption will be made that similar factors are responsible for both, with the reservation, however, that the various mechanisms may be relatively more or less important in each type of dyspnea. All conclusions concerning the origin of the dyspnea of cardiac decompensation are subject to the above limitations.

1. Anoxemia. The rôle of anoxemia in the causation of dyspnea has for many years been considered an important one. There is no need to review here the vast amount of experimentation on animal preparations on which this conclusion is based; these data are presented, at least in part, in most of the standard text-books of phys-

iology. It is, however, necessary to review the data bearing on the rôle of anoxemia in the genesis of cardiac dyspnea because of attempts (141) which have recently been made to discount their importance. That anoxemia of the tissues exists in congestive failure is shown by a large number of observations. These include evidence that the venous blood oxygen tension is low, and that, therefore, the tissue oxygen tension must also be low. The direct measurements of Meyer (230) further show low tissue oxygen tension in patients with heart disease. Additional evidence is offered by the many observations proving the presence of an increased blood lactic acid content at rest and after exercise and also of an abnormally prolonged oxygen debt after exercise in patients with congestive failure. The fact that some decompensated patients store oxygen when exposed to air containing high concentrations of that gas also is additional evidence as to the presence of anoxemia. Finally, the low arterial blood oxygen saturation points to the presence of anoxemia in the tissues also.

That degrees of anoxemia such as commonly exist in patients with cardiac decompensation are of importance in the causation of dyspnea is conclusively shown by the response of such patients to the administration of air enriched with oxygen; in most instances there is an immediate and striking improvement in dyspnea. Clinical improvement under such circumstances is not due to an increase in cardiac output or a fall in venous pressure. The changes which occur in the venous blood oxygen tension (and, therefore, in the tissue oxygen tension) after a variety of therapeutic procedures are confirmatory evidence. The return toward normal of the venous blood oxygen tension as a result of these therapeutic measures is usually associated with improvement in dyspnea.

Many factors act to cause tissue anoxemia in cardiac decompensation; these may be grouped under two main headings: (1) decreased delivery of blood to the tissues and (2) the effects of pulmonary congestion. The latter acts to cause a lowering of the arterial oxygen saturation by edema in the alveolar walls and by impairment of the bellows function of the lungs. The impairment of pulmonary function is in turn an expression of various subsidiary factors. Reduction of the pulmonary respiratory space and increase in dead space are in themselves causes of dyspnea in that they prevent complete arterial-

ization of all the blood in the lungs. Increased rigidity and decreased distensibility of the lungs, and an intrapleural pressure which is less negative than normal also prevent normal pulmonary function.

Lactic Acid Metabolism. One of the manifestations of tissue anoxemia is the occurrence of abnormally large amounts of lactic acid in the blood of patients with congestive failure at rest, but more strikingly after exercise. The accumulation of increased amounts of lactic acid in the blood, and presumably in the tissues also, indicates a breakdown of the normal mechanisms involved in the oxidation of that substance. It is known that the brain itself produces lactic acid (218), and there is evidence to indicate that interference with the normal rapid oxidative destruction of lactic acid produced in the respiratory center is a fundamental cause of dyspnea. This concept has been elaborated by Gesell (115). It is also highly probable that the presence of very high concentrations of lactic acid such as occur in the blood of cardiac patients after exercise may result in the diffusion of that substance into the respiratory center, with the consequent appearance of hyperpnea and dyspnea.

2. *Abnormal Stimuli from the Lungs.* It has been shown in animal experiments that hyperpnea results from a variety of reflex stimuli from the lungs. Intrapulmonary factors making for such stimulation are abnormal rigidity of the lungs, which activates the Hering-Breuer reflex, and simple congestion, which activates the reflex first demonstrated by Churchill and Cope. The nerve pathways involved in these reflexes are present in all individuals; in cardiac decompensation changes occur within the lungs resulting in the transmission of afferent impulses to the brain which evoke a motor response, i.e., hyperpnea. Perception by the patient of this increased respiratory activity may result in a sensation of dyspnea. There is no evidence to indicate that the congestive changes in the lungs result in dyspnea by giving rise to afferent impulses directly to the sensorium. The importance of reflex mechanisms in causing the sensation of dyspnea in patients with chronic congestive failure is still a matter for conjecture. In any event the rôle of reflexes in the genesis of dyspnea is secondary to that of the pathological congestion in the lungs.

3. *Increased Blood Volume.* The increase in blood volume which occurs in congestive failure results in an increase in the amount of

blood in the lungs, thereby exaggerating the pulmonary congestion which is due to other factors. Diuresis which decreases the blood volume frequently results in a striking improvement in dyspnea. The possibility, however, that at least part of the improvement following diuresis is due to dissipation of edema of the alveolar walls cannot be ruled out.

4. *Impaired Heat Dispersion.* A much neglected cause of cardiac dyspnea is alteration of the heat dispersal mechanisms due to a

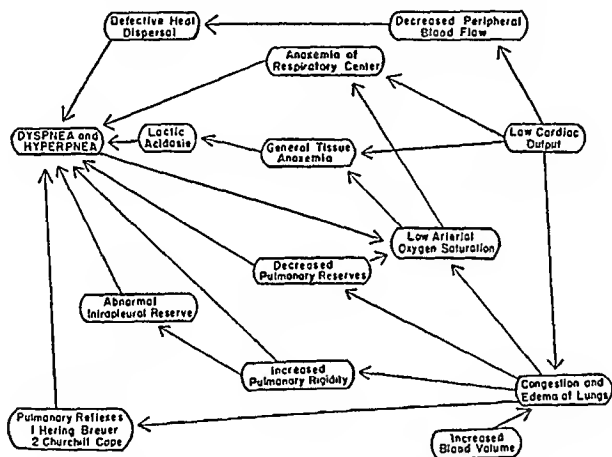


FIG. 3. THE MECHANISM OF DYSPEA IN CONGESTIVE HEART FAILURE

decreased flow of blood to the periphery. The previously discussed observations of Steele on the occurrence of a low skin temperature in spite of a high rectal temperature, and the findings of other observers that the insensible perspiration is low in cardiac decompensation strongly indicate the existence of this factor. The hyperpnea which is a response to the inability to disperse normal amounts of heat via the skin in cardiac patients must contribute to the dyspnea they experience. The fact that many severely decompensated patients claim to experience relief of dyspnea as a result of removing bed

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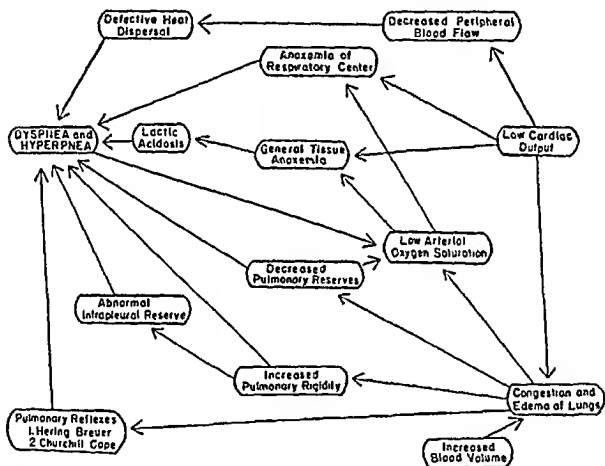


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clothes and being placed in front of an open window is in harmony with this concept.

5. *Blood Carbon Dioxide.* Normal or low carbon dioxide content of the arterial blood is the rule in uncomplicated congestive failure. Low arterial blood carbon dioxide levels are the result of blowing off of carbon dioxide by cardiac patients in whom dyspnea is the result of mechanisms other than carbon dioxide acidosis. Changes in blood carbon dioxide in cardiac decompensation are the result, rather than the cause of hyperpnea.

6. *Conclusions.* It must be concluded that cardiac dyspnea, like the previously discussed cyanosis and edema, is due to the operation of a multiplicity of factors. The inability of investigators to correlate exactly changes in physiological measurements with the degree of dyspnea is due not only to the fact that dyspnea is a sensation, and hence unmeasurable, but also to the fact that the various factors in the production of dyspnea vary in importance from patient to patient.

D. ORTHOPNEA

Orthopnea, like dyspnea, is a symptom and analysis of the pathogenesis of the former is subject to the same above discussed limitations as the latter. Orthopnea has been classified clinically as "orthopnea of necessity" and "orthopnea of choice." The terms "orthopnea of necessity" and "orthopnea of choice" merely distinguish degrees in the feeling of urgency to sit up experienced by patients, i.e., degrees of orthopnea itself.

The severity of orthopnea varies considerably from patient to patient although it parallels in a general way the degree of dyspnea. In its milder form the sensation is described as "discomfort in the chest," a "slight choking sensation" either in the throat or in the chest, a "smothered feeling," etc. on lying down. The time of onset of the sensation after assuming the recumbent position varies a good deal; in some instances the sensation appears immediately but does not become unbearable for some minutes, and in others it may appear only after a period of five to ten minutes and never becomes unbearable. In general, the more severe degrees of orthopnea are associated with a more nearly immediate onset of the sensation and are usually accompanied by a rapid increase of cyanosis of the face on lying flat.

Consideration of the factors responsible for the phenomenon of orthopnea must necessarily commence with a discussion of the cardiovascular and pulmonary changes which occur when the patient passes from the recumbent to the upright position. Physiologists have theorized concerning these changes in the thorax and elsewhere in the body. For instance, Sahli (293) regarded orthopnea as an expression of the fact that the upright position made possible a more efficient action of the respiratory muscles, and that pooling of the blood in the lower portion of the body relieved both cerebral and pulmonary congestion. The validity of such theoretical considerations, unsupported by observation, is not established. Fortunately, since the time of Sahli and his contemporaries a large volume of exact physiological data bearing on this problem has accumulated.

1. *Changes in Blood Flow Through the Lungs.* Lindhard (202) first pointed out the decrease in minute volume blood flow through the lungs of some normal individuals when the upright position is assumed. Subsequently other observers (66) (75) (77) (97) (155) (198) (286) (332) corroborated this finding. Grollman (129) and Schellong and Heinemeier (296), however, attacked the validity of the methods used by these investigators and claimed that experiments with Grollman's method revealed no such change. Subsequently Nylin (243) reviewed Grollman's data and concluded that they actually did corroborate Lindhard's original conclusions. In addition, a number of authors (28) (41) (98) (188) (243) (298) (303) (329) using Grollman's method and others (121) (329) using Gladstone's (121) modification also found a decrease in the minute volume blood flow through the lungs in the upright position. The validity of these observations seems to be established, at least for normal individuals. A. V. Bock and his co-workers (97), (198) regarded this change as of importance in the genesis of orthopnea in that it might tend to diminish pulmonary congestion. However, because of the fact that the data on changes in blood flow through the lungs resulting from change in position in patients with congestive failure are so fragmentary (28) (41) the concept of Bock and his co-workers cannot be regarded as established. Indeed, in view of the known decreased responsiveness of the cardiac output of patients with congestive failure to other stimuli, such as an elevated venous pressure, there is reason to suppose

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that variations in cardiac output in response to changes in position might be less striking in such patients than in normal individuals, or even completely absent.

2. *Altered Pulmonary Dynamics.* Haldane, Meakins and Priestley (134) found that normal subjects tended to become anoxicemic when they assumed the recumbent position. They ascribed this change to less effective pulmonary ventilation in that position. Subsequently Calhoun, Cullen, Harrison, Wilkins and Tims (49) confirmed their observations both in normal subjects and in patients with heart disease and added the significant finding that the arterial blood oxygen saturation is lower in recumbency. All available evidence indicates the validity of the original concept of Haldane, Meakins and Priestley (134) concerning the reason for the onset of anoxemia on lying flat. Rubow (292) in 1909 showed that assuming the recumbent position resulted in a decrease in the total pulmonary capacity. Hamilton and Morgan (137) and Hurtado and Fray (166) confirmed this finding. A decrease in mid-capacity occurs (31) (42) (57) (166) (292) and the reserve air is also diminished (42) (166) (348). The residual air may be diminished, according to Hurtado and Fray (166), or essentially unchanged, as Hamilton and Morgan (137) found. All authors are agreed that the vital capacity is usually lower in the recumbent position (42) (49) (55) (56) (166) (273), although Ernstene and Blumgart (90) and Hamilton and Morgan (137) found the changes to be rather small. It is apparent that the changes in the lungs themselves which occur as a result of lying down make for decreased pulmonary efficiency. The intrapleural pressure also changes, becoming less negative. This was first pointed out by Aron (11) in 1891 and subsequently confirmed by Christie and McIntosh (57) and Prinzmetal and Kountz (271).

The pulmonary changes were regarded by Haldane, Meakins and Priestley (134) and by Livingstone (203) as due to a shift in the position of the diaphragm. Hamilton and Morgan (137) could not confirm this finding; they ascribed the lung changes to a variation in the amount of blood in the thorax in different positions. However, their conclusions, based on the observation that placing tourniquets on all the extremities resulted in an increase in vital capacity of three to eleven per cent, must be considered too sweeping. Additional evi-

dence against their concept is the fact that the pattern of the pulmonary changes which occur as a result of recumbency is not the same as that of congestive failure and that, therefore, the changes which occur in recumbency are not due to congestion. In congestive failure the total and mid-capacity are diminished markedly and the residual air relatively or absolutely markedly increased. In the recumbent position the changes in total capacity are slight and the residual air is absolutely or relatively decreased. Whatever the mechanism of the pulmonary and intrapleural changes, they serve to explain the decreased arterial blood oxygen saturation which occurs, even in normal subjects, as a result of lying flat. This change in the arterial oxygen saturation is a factor in the genesis of orthopnea.

3. Increased Venous Pressure. The importance of an elevated venous pressure in the genesis of orthopnea was pointed out by Ernestene and Blumgart (90). Their observation that merely flexing the head, thereby decreasing the venous pressure in the respiratory center, resulted in relief of orthopnea, in spite of the absence of any change in the vital capacity or position of the chest, has been corroborated by Calhoun, Cullen, Harrison, Wilkins and Tims (49), although these authors found no evidence of slowing of the blood flow through the brain in the recumbent position. Altschule and Blumgart (6) recently had the opportunity of re-investigating this point in a patient with marked engorgement of the face and scalp veins due to tricuspid stenosis. It was found that positions in which collapse of the engorged veins of the face and scalp occurred were associated with relief of orthopnea, whereas engorgement of the face and scalp veins was always associated with the presence of that symptom. The observation of Calhoun et al. (49) that placing a tourniquet around the neck, while it did cause increased respiratory activity in some cases, did not cause dyspnea is difficult to interpret. Robertson and Fetter (283) recorded the observation that venesection reduces the venous pressure and relieves orthopnea as well.

4. Increased Cerebrospinal Fluid Pressure. Increased cerebral venous pressure, as was discussed previously, results in an elevation of cerebrospinal fluid pressure. Harrison (151) has recently stated that the increase in spinal fluid pressure is a primary factor in the genesis of orthopnea. In his experience lumbar puncture relieved

dyspnea without change, in some cases, of the venous pressure as measured in the arm. However, since the skull is an inelastic structure, changes in pressure in the veins is reflected in cerebrospinal fluid pressure and vice versa. It is, therefore, difficult to state whether the increase in cerebrospinal fluid pressure which occurs as a result of elevation of the venous pressure is in itself a cause of orthopnea.

5. *Mechanical Factors.* It is more than likely that mechanical factors are of some importance as causes of orthopnea. The pressure of the abdominal viscera, or ascites or both, on the diaphragm when the patient is recumbent may interfere with its free movement to some degree. The frequent occurrence of orthopnea in patients with

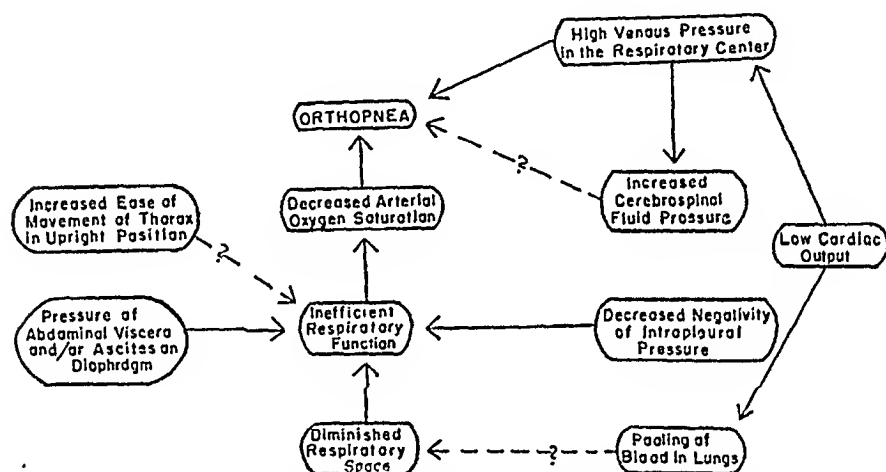


FIG. 4. THE MECHANISM OF ORTHOPNEA IN CONGESTIVE HEART FAILURE

ascites due to other causes, such as cirrhosis or neoplasms, favors this view. The somewhat greater ease of movement of the chest muscles in the upright position is not by itself sufficient to explain orthopnea; orthopnea is not seen in patients with thoracic muscular paralyses or with muscular weakness due to myasthenia gravis. The greater ease of thoracic movement in the upright position may, however, be a contributory factor in the genesis of orthopnea.

6. *Conclusions.* It appears to be valid to regard orthopnea as due to the combined action of a number of factors; additional factors may be present but still unrecognized. The importance of the various factors evidently varies considerably in different patients.

E. EFFECT OF THERAPY ON SIGNS AND SYMPTOMS AND ON CARDIOVASCULAR PHYSIOLOGY. STATUS OF THE "ADEQUATELY" TREATED CARDIAC PATIENT

1. *Effect of Digitalis.* The numerous studies on the effect of digitalis on the cardiac output may be summarized as follows: Digitalis causes an increase in the cardiac output of almost all patients with congestive failure and rapid auricular fibrillation and in some patients with congestive failure and no arrhythmia. The effectiveness of digitalis seems to diminish during successive bouts of cardiac decompensation, apparently more rapidly in patients with regular rhythm than in those with auricular fibrillation. In most of the cases in which the cardiac output increases as a result of digitalization, a complete return to normal values is not secured; in some instances the increase obtained is probably not the most important factor in causing clinical improvement in patients treated in the hospital. When clinical improvement occurs as a result of the administration of digitalis, the rise in cardiac output is appreciable and the arteriovenous oxygen difference becomes more nearly normal; direct measurements on the venous blood oxygen tension reveal increases, implying an increase in tissue oxygen tension.

As improvement becomes apparent following digitalis therapy the venous pressure falls and increases in total lung volume and its subdivisions are observed. Simultaneously the blood volume returns toward normal and evidences of increased blood destruction, viz., increases in bile pigment in stools and of urobilin in urine, become evident.

However, in the majority of instances the measurements obtained after improvement due to digitalization occurs are still abnormal to a greater or a lesser degree. Complete physiological recovery is extremely rare. These findings are in complete agreement with clinical experience. Digitalis clinically causes some degree of improvement in the status of patients with congestive failure but following the first bout of failure almost all patients are cardiac invalids for the rest of their lives. A few exceptions are those patients in whom cardiac failure has occurred as a result of thyrotoxicosis or some paroxysmal arrhythmia and those extremely rare cases in which failure due to

acute rheumatic fever, or to the immediate effects of coronary occlusion, disappears, not to reappear for many years, if at all.

The results of laboratory experiments on the effects of digitalis in congestive failure support the clinical impression that digitalis, though extremely useful, by itself does not usually control congestive failure. Indeed in many instances the administration of that drug is not the most important of the various therapeutic measures employed.

2. *Effect of Diuretics.* Studies on the cardiac output following the administration of all the commonly used diuretics has shown that no change in the minute volume output of the heart occurs (111) (281) (326). Nevertheless striking improvement in edema, and even in cyanosis and dyspnea may occur. These are to be ascribed to the diminution in blood volume, and secondarily in venous pressure, and to changes in the pulmonary physiology which follow disappearance of congestion. According to studies now available, diuretics do not improve the fundamental defect in cardiac disease, the low cardiac output, but do effect symptomatic improvement by favorably affecting certain other physiological abnormalities which supervene in congestive failure.

3. *Effect of Oxygen Therapy.* The cardiac output is apparently not significantly affected by the administration of air containing high concentrations of oxygen (15) but changes in the arterial blood oxygen saturation, with a consequent improvement in the tissue oxygen tension do occur. Simultaneously the abnormally high blood lactic acid level may be restored to or toward normal and the lowered carbon dioxide dissociation curve, indicative of a tendency toward acidosis, becomes elevated to normal. If diuresis occurs, the venous pressure falls. A lowering of the venous pressure level also occurs as a result of the relief of hyperpnea.

It must be concluded that oxygen therapy, like the diuretics usually does not influence the fundamental defect in cardiac output, of congestive failure, but affords symptomatic improvement through its action on the physiological abnormalities which supervene.

4. *Effect of Venesection.* Venesection may cause immediate improvement in edema and cyanosis, and what later may even be followed by a permanent improvement. This can be due to a change in cardiac output, or to a change in the pulmonary physiology, or to a change in the tissue oxygen tension.

put of the heart is temporarily diminished because of the transitory decrease in venous return. However, a decrease in blood volume occurs, with a fall in venous pressure and decrease in congestion of the lungs, sufficient to explain the clinical improvement. The improvement in dyspnea is due not only to cessation of reflex dyspnea which results from decreased pulmonary congestion, but also to decreased anoxemia due to an increase in vital capacity, and improvement of the arterial blood oxygen saturation (126). Similarly such diuresis as may occur is due not only to the lowered blood volume, which causes a withdrawal of fluid from the tissues (200), and the lowering of the venous pressure, but also the above described alleviation of anoxemia. The relief of orthopnea following venesection can also be shown to be due to the changes which occur in the various factors responsible for it.

5. *Effect of Rest.* Bed rest not only serves the obvious purpose of cutting down activities to or toward a level which the heart can support but is also important in that it enables many patients who are in a state of continuous oxygen debt to discharge that accumulated debt. Patients who exhibit an elevated blood lactic acid level show significant decreases in blood lactic acid, if improvement follows marked limitation of activity.

6. *Limitation of Fluid and Salt.* This clearly does not influence the cardiac output, but acts to inhibit the accumulation of extracellular fluid.

7. *Conclusions.* In general, it must be concluded that improvement in physiological and in clinical status following digitalis therapy is associated with a partial return of cardiac output toward normal, whereas the favorable response to other forms of therapy such as diuretics, oxygen, venesection, bed rest and limitation of fluids and salts is due mainly to correction of some of the secondary effects of a lowered output.

It is possible that in some instances an improvement in cardiac function may occur as a result of bed rest, oxygen therapy, venesection, the use of diuretics, and limitation of fluids and salt. These various procedures tend to influence cardiac function favorably by combating anoxemia, either directly as in the case of oxygen, or by diminishing pulmonary congestion and thereby causing an

increase in arterial oxygen saturation, as in the case of the other above-mentioned forms of therapy. Although there are available no physiological data concerning this point, certain clinical considerations indicate that in some cases this concept must be invoked to explain improvement. All physicians have seen patients who enter the hospital in a state of severe decompensation and whose course under observation is rapidly downhill. In such instances the use of oxygen therapy and venesection may cause a striking immediate improvement, which persists even after the use of oxygen is discontinued. Apparently a vicious circle exists in these patients, extreme congestive failure giving rise to severe anoxemia causing a decrease in cardiac output which serves to further aggravate the anoxemia. Under such circumstances it is probable that forms of therapy which diminish anoxemia cause some rise in cardiac output, thus interrupting the vicious circle.

8. *Physiological Status of the "Adequately" Treated Cardiac Patient.* A group of patients were studied in order to determine the physiological status of the patient with chronic cardiac decompensation in whom the greatest possible improvement in symptoms was secured by treatment in the hospital. The following table (Table III) includes the findings in eight unselected patients with chronic cardiac decompensation at the time of discharge from the hospital. These patients received the benefit of complete bed rest for several weeks, limitation of fluids and salt, full doses of digitalis, adequate diuresis with mercurial preparations, and in a few instances venesection or oxygen therapy as well. It is evident that striking symptomatic improvement had occurred—i.e., there was no edema and at most minimal dyspnea at rest. However, evidence of the persistence of an abnormal cardiovascular physiology was shown by the somewhat decreased cardiac output, tendency toward an increased respiratory rate and minute volume, a slight decrease in arterial blood oxygen saturation, a lowering of the vital capacity, a prolongation of the pulmonary circulation time, and, in one patient, slight elevation of the venous pressure. These findings corroborate and extend the observations of other authors who have studied the cardiovascular system after recovery from congestive failure (144) (183) (184) (188) (282) (316) (317). The persistence of abnormal physical findings such as cyanosis, cardiac

TABLE III

CASE	AGE	SEX	RESPIRATORY RATE	RESPIRATORY VOLUME	VITAL CAPACITY	CARDIAC OUTPUT	NASAL METABOLIC RATE	PULSE RATE	CIRCULATION TIME	VENOUS PRESSURE	ARTERIAL BLOOD OXY-GEN SATURATION	ARTERIAL PRESSURE	DIAMETER OF HEART AND DIAMETER OF CREST BY X-RAY	CYANOSIS	DYSPNEA AT REST	DYSPNEA ON EXERCITION	RATES	ENLARGEMENT OF LIVER	EDEMA	DIAGNOSIS
1	40	F	14	liters per sq. m. 4.6	liters per sq. m. 1.4	liters per sq. m. 1.6	-14	61	32	8.3	91	135/65	17.6/27.0	++	0	++	0	3	0	Rheumatic heart disease. Mitral stenosis. Auricular fibrillation
2	66	M	18	4.6	1.9	1.8	-9	75	22	2.3	91	135/85	17.1/30.5	+	Minimal	++	0	0	0	Coronary thrombosis
3	38	M	12	3.7	1.6	1.6	+1	51	42	10.6	89	120/70	19.9/29.5	++	0	+++	0	5	0	Rheumatic heart disease. Mitral stenosis. Auricular fibrillation
4	48	M	19	5.1	1.3	1.8	-1	72	33	5.4	91	120/70	18.6/29.2	+	+	++	+	0	0	Rheumatic heart disease. Mitral stenosis
5	62	M	19	3.6	1.4	1.9	+1	62	23	3.2	91	180/90	16.9/29.6	+	Minimal	++	+	3	0	Hypertensive heart disease
6	36	F	16	4.0	1.3	1.8	±0	41	22	13.0		120/80	15.9/23.6	+	0	+++	+	3	0	Rheumatic heart disease. Mitral stenosis, aortic stenosis. Auricular fibrillation
7	67	M	12	4.1	1.7	1.6	+8	76	22	5.2		124/100	16.6/29.7	+	0	++	Rare	3	0	Coronary thrombosis
8	49	M	17	3.7	0.9	1.6	-3	43	58	8.9		120/70	20.1/29.3	+	+	+++	+	6	0	Rheumatic heart disease. Mitral stenosis. Auricular fibrillation

enlargement, and hepatic enlargement in the cases here reported is to be noted. *The symptoms of dyspnea, orthopnea and edema were controlled but the cardiovascular physiological measurements in each case failed to return to normal values.* Following discharge from the hospital all of the patients, except one who died, returned with fully developed cardiac decompensation within a year in spite of the fact that their activities were markedly limited. It is clear that the improvement they experienced as a result of their hospital stay was due to the treatment of the secondary consequences of an inadequate cardiac output as well as to increase in the minute volume output of the heart, which, however, did not reach normal levels even with the patient at rest in bed. This concept explains not only the physiological findings, but the clinical course in chronic cardiac decompensation. Some patients, especially those with mild or moderate cardiac decompensation find a level of activity at which they can avoid the signs and symptoms of congestive failure without much active treatment. In other cases, particularly those with more severe degrees of failure, continuous treatment, such as the frequent use of diuretics and repeated hospitalization is necessary in spite of marked limitation of the patient's activity.

The implications of this concept are favorable to the employment of total thyroidectomy in the treatment of selected cases of congestive failure (36) (37) (38). Since it is frequently impossible to restore the cardiac function to normal in the treatment of congestive failure, a logical procedure is to decrease the metabolic needs of the body to a level which the impaired cardiac function can support.

F. "BACKWARD" FAILURE. "FORWARD" FAILURE. A GENERALIZATION CONCERNING THE PATHOGENESIS OF THE SIGNS AND SYMPTOMS OF CONGESTIVE FAILURE

It is evident that cardiac decompensation is associated with a large number of complexly interrelated bodily changes. That other changes, as yet unrecognized, may occur is very likely.

A low cardiac output is the rule in cardiac decompensation. Some authors have reported normal values for minute volume output in some patients with congestive failure. In such cases, however, the reported basal metabolic rates have ranged from plus fifteen to plus

eighty per cent. It is clear that under these circumstances the cardiac output is low in proportion to the metabolic needs of the body. Many patients with congestive failure have an elevated venous pressure. The normal response to an elevated venous pressure is an increase in cardiac minute volume output; decompensated patients do not exhibit this response. The widespread physiological changes found in congestive failure are all probably secondary to a cardiac output which is low in relation to the metabolic needs of the body and to the venous return. It must be remembered, however, that some of these secondary changes may overshadow the lowering of the cardiac output as the direct cause of one symptom or another in some patients. Thus in some instances intrapulmonary changes may be more important in the genesis of dyspnea, or lowering of the plasma protein more important in the genesis of edema than anoxemia due to an inadequate cardiac output. The low cardiac output of congestive failure may give rise to the signs and symptoms of cardiac decompensation in many different ways. All or almost all of these different mechanisms are present in all patients with congestive failure. One mechanism or another may predominate in a given patient, or several may be equally important.

Any theory which attempts to explain the origin of all the signs and symptoms of congestive failure as *directly* due to "forward" failure (low cardiac output) or "backward" failure (increased peripheral and pulmonary venous pressures) is clearly inadequate. Back pressure can occur only if the blood fails to go forward; similarly failure of the blood to go forward must result in back pressure, provided the volume does not decrease. An additional criticism of both theories lies in the fact that both neglect the chemical changes which occur in cardiac decompensation which are potent factors in the genesis of various signs and symptoms.

A valid generalization concerning the origin of the signs and symptoms of chronic congestive failure is that they are due to a summation of the effect of submaximal or even minimal changes in a multiplicity of complexly interrelated factors and that the degree of change in each of these factors, and consequently their importance, varies from patient to patient. The fundamental defect, however, is a cardiac output which, in relation to the metabolic requirements of the body

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THE MAMMALIAN BLOOD PLATELET IN HEALTH AND DISEASE¹

LEANDRO M. TOCANTINS

Department of Medicine and Laboratories of Pathology, Jefferson Medical College and Hospital

CONTENTS

INTRODUCTION.....	156
PHYSICOCHEMICAL PROPERTIES.....	157
Dimensions.....	157
Volume.....	158
Color.....	159
Form.....	159
Structure.....	160
Pseudo-platelets.....	162
Comparative morphology.....	164
Agglutination.....	165
Physical alterations.....	168
Chemical composition.....	171
Respiration.....	173
Electrical properties.....	174
Pharmacological action.....	175
THE PRODUCTION OF PLATELETS.....	176
Origin of platelets.....	176
Distribution of megakaryocytes.....	183
Origin of megakaryocytes.....	184
Sites of platelet production.....	187
Mechanism of production.....	188
Rate of production.....	190
Abnormal thrombopoiesis.....	191
THE DISTRIBUTION OF PLATELETS.....	194
In the tissues.....	194
In the vessels.....	195
Numerical variations. Physiological.....	197
Numerical variations. Pathological.....	208
THE UTILIZATION OF PLATELETS.....	221
Platelets and physical properties of the blood.....	221
Platelets and hemostasis.....	229
Platelets and thrombosis.....	232
Platelets and resistance to infection.....	235
THE DESTRUCTION OF PLATELETS.....	242
BIBLIOGRAPHY.....	243

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INTRODUCTION²

Divergent findings abound in the literature on blood platelets, and are largely the result of differences in methods of examination. When compared with other morphological elements, the platelet is so small and its existence outside of the body so ephemeral that it is not surprising that many methods used to examine it yield gross qualitative and quantitative errors. For example, the enumeration of platelets, as currently carried out on cutaneous blood, has, for reasons to be given further on, only a limited value. To discard, however, all the evidence accumulated with the employment of these methods, would be discarding approximately three-fourths of the available knowledge on variations in the number of platelets. This evidence, therefore, will be analyzed along with the rest, but it should be kept in mind *that many of the facts thus brought forth demand reëxamination* with more improved methods, before being finally accepted as true.

In spite of valuable efforts in that direction, little as yet of a definite nature is known regarding the physiological variations in the platelets of man and other animals. To this fact must be attributed much of our failure to understand and properly interpret disorders of functions in which platelets are involved.

It is preferable to think of the platelet not as a cellular element but as a transition between the microscopically amorphous elements of the blood (proteins, fats, etc.) and definitely cellular constituents such as erythrocytes or leukocytes. The mode of origin of the platelet from the megakaryocyte suggests that it is the product of a cell rather than a cell in itself. As Howell and Donahue (310) have expressed it, platelets represent a solid secretion from a unicellular gland, the megakaryocyte. Moreover, in the performance of their functions, platelets are, unlike cells in general, nearly always irreversibly altered or destroyed. These are essential points to keep in mind when analyzing, among other things, the relationship between the number of circulating blood platelets and the number of megakaryocytes, differences in platelet content between arterial and venous blood, and

² For an account of the facts surrounding the discovery, and development of knowledge of platelets, the reader is referred to the following sources: Duperié (1878), Osler (1883), Howell (1884), Muir (1891), Lilienfeld (1892), Aynaud (1909), Hayem (1923).

the significance of quantitative and qualitative variations of the platelets in disorders of hemostasis.

Even this conception of the platelet as more akin in many ways to the amorphous than to the cellular elements of the blood does not hold in every respect. Under certain conditions it seems that platelets may be used for a short length of time, to return to the circulation before any changes in their structure have taken place. Masses of platelets may form around a foreign body or a portion of a vessel, with subsequent detachment of the mass, which floats off into the circulation to break up again into its constituent parts (76) (185) (16). Under these circumstances the platelet would behave more like the erythrocyte; it would perform a function, and not be consumed, or irreversibly altered while performing it. Perhaps a conception of this dual nature of platelets will be of help in the interpretation of their changes and elucidation of their functions.

PHYSICOCHEMICAL PROPERTIES

Dimensions. In man the length of platelets varies between 1 and 5 micra (Table 1), most forms measuring between 2.4 and 3.2 micra. In dogs, platelets are a little larger, forms between 3 and 6 micra in length not being rare. The large percentage of small platelets ($1\ \mu$ or less) observed on cutaneous blood when Tyrode solution is used as a diluent (301), represents products of platelet disintegration induced by contact with tissue juices before fixation (517). There seems to be no change in size with age (281). There are no measurements available on the thickness of platelets; judging from their appearance and the volume occupied by a given number, the thickness of normal platelets is perhaps between 0.5 and $1\ \mu$. Increase in size is one of the outstanding changes observed under abnormal conditions. Giant forms, measuring 20 – $25\ \mu$ in length, may be observed during the initial stages of regeneration of platelets after natural or artificially induced thrombopenias (626). In the blood of a patient with Hodgkin's disease, Bunting (107) frequently found megaloplatelets, one of which measured approximately $50\ \mu$ in length. The largest platelet that has come under our observation was in the blood of an individual with a severe hyperchromic anemia (Plate 1, Fig. 12); it measured $48\ \mu$ in length and appeared to be undergoing segmentation. In wet counting

chamber preparations, megalo-platelets appear as long, refractile bodies, resembling hyaline casts (Fig. 1 B). The shape of these giant forms suggests their likely origin as pinched off portions of cytoplasmic processes. In dry stained smears their large size and heavy granulation distinguishes them sharply from normal forms.

Volume. The average volume percent of platelets in the blood of man is 0.49 (range: 0.35–0.67%) (644). Horwitz (301) found the figures for adult males 0.39%, females 0.39%, children 0.45%. In the dog the average percent platelet volume is 1.04% (644). In 6 dogs with a mean venous platelet count of 460,000 (range: 360–550), a mean percent platelet volume of 0.96% (range: 0.70–1.25%) was found (634). In the rabbit the mean percent platelet volume is 0.53%

TABLE 1

Differential count of normal platelets according to their approximate length

	1.6 μ OR SMALLER	3.2 μ	4.8 μ	6.4 μ	8.0 μ	9.6 μ	REMARKS
Man.....	13.2	69.7	15.3	1.4	0.4		Average percentages from 6 counts on the venous blood of 5 normal men.
Dog.....	9.4	55.5	28.0	5.33	1.48	0.29	Average percentages from 8 counts on the venous blood of 4 normal dogs.

Diluting solution: Sodium citrate and formalin.

Method: (Tocantins, 1937) (630).

(range: 0.4–0.72%) (644). These figures apply to venous blood. In arterial blood the number of platelets is generally higher and, therefore, the percent volume of platelets should be higher. Comparative volumetric determinations on blood from continuous arteries and veins in normal dogs did not reveal important differences in the individual size and volume of platelets (634). As platelets go through capillaries, they are often seen to fragment into smaller forms (634); such fragmentation may be observed also in blood diluted with Tyrode solution (87) (277) (325) (382). Platelet counts on venous blood may thus appear equal to, or higher than those on arterial blood, even though the total platelet volume percent in the latter be equal or greater than that in the former. Under physiological conditions there is a

man these forms are more commonly observed during periods of abnormal platelet regeneration. The shape most commonly assumed by platelets in the circulating blood of man and dog is that of an oval disc or lentil (76). Aside from these forms, there are numerous others, such as those described by Deetjen (145) and Hayem (286), which represent, mainly, alterations undergone by the platelet outside of the body. The altered platelet may assume many shapes even when in isotonic solutions; its edge may appear irregular and full of spine-like processes, some of which may exceed two or three times the main diameter of the platelet (Plate 1, Fig. 10, B). The shapes assumed by platelets in smears of unfixd blood allowed to dry slowly, are due mainly to surrounding physical forces, such as pressure and contact with glass, drying, adhesion and pressure of other cells, etc.

Structure. In dry stained smears of imperfectly fixed blood, platelets appear divided into 2 zones: the clear hyalomere, and the chromomere made up of granules. This separation into 2 zones is probably caused by changes in the granular material within the platelet after leaving the vessel. In the intact circulating platelet, the granular material is distributed finely and evenly throughout the hyaline portion (29). Its accumulation into a group of coarse granules surrounded by a hyaline zone is a result of precipitation and agglomeration of this material. When blood is collected directly into a fixing fluid (formalin, osmic acid), the platelets are instantaneously fixed; subsequent staining with Giemsa will show the stain evenly distributed through the body of the platelet, with no sharp zone of separation between hyalomere and chromomere. Treatment with other stains (Saffranin, gentian violet, brilliant cresyl blue) yields identical results (Plate 1, Fig. 8, C, D, E, F). If the diluted Giemsa stain is applied to the smear for 30' to 60', the fine granules may be made out (Plate 1, Fig. 8, C); but if the staining is prolonged (2-3 hours), the platelet is stained an even reddish violet. The appearance of the platelet varies further, according to whether the smear was made before or after the blood was fixed and stained. There is no better description of the appearance of platelets when stained with Wright's stain than the words of Wright himself (681): "They consist of a hyaline blue staining substance in which are imbedded closely set, minute, red to purple staining granules. In deeply stained prepara-

tions, this hyaline ground substance may not be apparent. The red to purple staining granules may be aggregated in a more or less sharply outlined mass in the central part of the platelet so as to suggest a nucleus surrounded by a hyaline cytoplasm. In some platelets a clear, unstained, more or less sharply outlined vacuole-like area may be seen in the midst of the granules. The structure of the blood platelets, and especially the peculiar color taken by the granules within them, are very characteristic and sharply distinguish them from the other elements of the blood."

Macroplatelets found in the blood in periods of abnormal blood regeneration take a deeper stain and often do not show, even in slowly dried preparations, a clear separation between hyalomere and chromomere (Plate 1, Fig. 11). The granules are coarse and distributed evenly throughout the platelet. If these platelets represent younger forms, their appearances on dry stained smears may result from the fact that, as Baar and Szekely (32) have demonstrated, young platelets take longer to disintegrate than older forms. Abnormally staining platelets have not apparently been through the last steps in differentiation from megakaryocytes, namely, the subdivision of the coarse into fine granules and their agglomeration into small masses ("Felderung"). Rows of giant platelets with granules clumped into small groups preparatory to the fragmentation of the cytoplasmic mass into smaller platelets ("pearl chains,") (581) may be observed in the blood during periods of active platelet regeneration (681). Macroplatelets with coarse granules may either represent forms fragmented from areas close to the nucleus after most of the cytoplasm has been stripped of fully differentiated material or come from incompletely differentiated megakaryocytes (megakaryoblasts, lymphoid megakaryocytes).

There is evidence that the granules of blood platelets may be derived from nuclear material. In the liver of pig embryos, the cytoplasm of megakaryocytes and the granules of platelets stain with Toluidin Blue (which only stains nuclei and the chromomere of platelets) (67). Comparison of megakaryocytes of different ages gives one the impression that material is cut off from the nucleus and that this then gradually breaks up into fine granules with accompanying change in staining reaction (169) (Plate 1, Fig. 6). At first these masses break-

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ing off from the nucleus stain like the nucleus itself, and are shaped very much like the Nissl's bodies of nerve cells (169). Eventually they break up into fine metachromatic granules that stain violet with the Wright, Giemsa or Dominici stains. The development of these granules seems to be associated with degenerative changes in the nucleus, though it is not possible by staining reactions to establish a direct relationship between platelets and nuclei of giant cells (169). The search for nuclear material in platelets by testing their response to Feulgen's reaction (specific for thymonucleic acid) has yielded either negative (550) or inconclusive results (131). The reaction is always negative but it is not possible to exclude the fact that the acid may be present in platelets in a dilution beyond the limits of visibility of the reaction (131). According to Deetjen (145) and Koppsch (365), platelets have a nucleus with chromatin network which may be demonstrated at high magnifications ($\times 3200$) and if special precautions are taken. Dekhuysen (148) considered platelets as cells endowed with all their attributes and called them "thrombocytes." Those who maintain that the chromomere of platelets represents a nucleus, point out that it stains with Giemsa fluid, like the nuclei of protozoa, and that some platelets show mitotic figures (496). Against this view are the following facts: 1) the chromomere of platelets cannot be stained by hematein or any anilin dye (25) (169); 2) methyl green, a nuclear elective dye, does not stain it; 3) non-nuclear material such as the azurophil granules of lymphocytes and monocytes also stain with Giemsa's liquid; 4) the azurophil granules of platelets are spread finely throughout its mass, if precautions are taken to fix it rapidly; 5) contrary to all nuclear material, the chromomere of platelets is traversed by ultra violet rays and is dissolved in acetic acid. No nucleus, inner bodies or nuclear membrane have been demonstrated in platelets (147) even by ultra violet light photography (577). This reviewer has never observed any platelets that could be strictly termed nucleated or in mitosis.

Pseudo-platelets. To know what *is not* a platelet is perhaps equally as important as to know what *is* a platelet. Some investigators are inclined to designate any cellular fragment as a platelet, regardless of its tinctorial characteristics. As Downey (169) pointed out, the blood

of normal animals does not show the great variations in morphology attributed to the platelet. Because platelets originate by a pinching off process from the cytoplasm of megakaryocytes, it does not follow that every extrusion or pinched off body from any cell is a platelet. Errors have been made more often by designating too many and diverse objects as platelets than by failure to recognise normal forms. This statement applies to observations made on fixed section material, smears of blood and, perhaps even more, to platelet counting on wet preparations. The impression is rooted in some minds that in counting platelets the greater the number found, the more accurate is the method employed. The characteristic appearance of the granules of rapidly fixed and properly stained platelets differentiates them from the hyaline bodies constricted off from the cytoplasm of lymphocytes and mononuclears (169). Under pathological conditions, as in the leukemias, these hyaline structures may be found in the blood. Besides lacking the staining affinities of platelet granules (Plate 1, Fig. 9) these structures are nearly always single and not in clumps, are round or oval, and seldom have the elongated appearance of giant platelets. The form and volume of the azurophilic substance of pseudoplatelets differ from true platelets and the presence of neutrophilic or eosinophilic granules reveals their leukocytic origin (115). In the simple thrombocytosis that follows splenectomy, for example, hyalin platelet-like bodies are not observed. While making smears of leukemic blood, the detachment of bits of cytoplasm from abnormally fragile leukocytes creates platelet-like bodies, which, however, stain identically with the cytoplasm of the immature cells. Birch (75) observed active budding and fragmentation of the cytoplasm of leukocytes in chronic myeloid leukemia, giving rise to pseudo-platelets. The increase in the number of platelets in chronic myeloid leukemia is probably due in part to inclusion of such forms in the count. The prolonged bleeding of some patients with chronic myeloid leukemia may be explained by the actual decrease in the number of true platelets and an increase in the number of pseudo-platelet forms (75). Under these conditions, counts on wet preparations should be supplemented by examination of stained blood smears. Pale microerythrocytes like those often found in the hypochromic, microcytic anemias,

or fragmentation products of these cells may be mistaken in wet unstained preparations for blood platelets. (For illustrations of pseudo-platelets, consult Watson (658).)

Pseudo-platelets may be observed in diseases accompanied by increased blood destruction, as malaria (253). Such forms may even be identified in sections of the spleen in malaria and leukemia and resemble basophilic erythrocytes and giant platelets (115).

Platelets have been mistaken for blood parasites and parasites for platelets. When Laveran described the asexual forms of the malarial parasite, it was generally thought that he had mistaken platelets for the parasites. The chromatin network of malarial parasites stains like the granules of platelets (14). There are close morphological tinctorial similarities between *Babesia* (piroplasma) and blood platelets, particularly when the latter have stood for sometime outside the body; they may then resemble the flagellate forms of piroplasmas for they seem actively motile and lash about in the same manner as the flagellum of *Euglena* (611). A few platelets when stained, even present the so-called nuclear dimorphism seen in trypanosomes. Students of Hematozoa have, from time to time, announced the discovery of blood parasites and later have found them to be only normal constituents of the blood (611). Bizarre platelet forms in the blood of patients with yellow fever were described during the New Orleans epidemic of 1905 as being the pathogenic agents of the disease (143).

Comparative morphology. It is generally agreed that platelets are found in the blood of all normal mammals. Even in the lowest order of mammalia, the monotremata, which have many of the structural characters of reptiles and birds, the platelets and erythrocytes are similar to those of other mammals (298). According to Aynaud and Pettit (30), it is possible to demonstrate platelets in the blood of birds, batrachials, reptiles and fishes, provided rapid manipulation and a special technique are employed. The blood of the salamander contains a non-nucleated colorless corpuscle which Eisen (190) observed to detach itself from the body of nucleated fusiform blood cells. These corpuscles are similar in structure and behavior to the platelets of mammalian blood (191) but are difficult to demonstrate by ordinary staining methods. In the blood of the crayfish (*astacus fluviatilis*) there are hyaline bodies identical with platelets in morphology,

fragility and agglutinative power (66) (615). Hayem (280) found his "hematoblasts" even in the blood of ovipara, and Robin (535) observed "globulins" in the circulating blood of fishes and batrachials. Platelets, as such, are perhaps present in all animals possessed of a circulatory system; they do not seem to exist in the body fluid of Echinodermata (615). In the blood of fishes, amphibians and reptiles, most red cells are nucleated, but there are a few smaller, non-nucleated forms (34) which correspond to the erythrocytes of mammalian blood. It is probable, likewise, that platelets are normally found in the blood of these animals, even if in smaller numbers than in mammalian blood.

According to Wright (681), Schridde (574) and Hartman (276), the thrombocytes of birds and the mammalian megakaryocytes are structurally and genetically the homologues of one and the same cell which circulated in the blood of extinct vertebrates. Wright (681) found the forerunners of the megakaryocytes to be circulating blood cells in the blood of embryonic guinea pigs. Reduction by saponin in the number of thrombocytes of birds leads to the appearance in the blood stream of giant cells believed to be the precursors of the thrombocyte of the bird (132), a phenomenon analogous to the occasional appearance of megakaryocytes in the peripheral blood of mammalia. The concept that the spindle cells (thrombocyte) of birds *functioned* as blood platelets was introduced by Bizzozero (77), who pointed out that, like the platelets of mammalian blood, thrombocytes played an important part in blood coagulation and thrombosis. Injection of an antithrombocyte serum into birds has produced purpura similar to that brought about by antiplatelet serum in mammals (389).

Agglutination. Agglutination of platelets may be precipitated by the least modification of the plasmatic equilibrium, as by addition of electrolytes or colloids or even by such physical changes as accompany the beginning of coagulation of the blood. Platelets suspended in citrated or oxalated plasma may be strongly agglutinated by a number of colloidal solutions of organic and inorganic compounds (gelatin, peptone, ovalbumin, casein, gum arabic, gum mastic, lecithin, mercury, silver, gold, platinum), by solutions of ricin, saponin or sodium taurocholate and of dyes such as eosin, saffranin, indigo carmin and trypan red (25). The greater the concentration of citrate or oxalate in the plasma, the less the agglutination. Platelets may be agglu-

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minated in incoagulable plasma by peptone, the effect on the platelets thus being distinct from that on the plasma. Heated or unheated water or ether extracts of heterologous or homologous muscle produce rapid agglutination of platelets; saliva, gastric and pancreatic juice, pepsin, milk and albuminous urine act in the same way, but bile has only a disintegrating action (25). Normal homologous serum from blood collected without contamination with tissue juices has very little agglutinating action. No isoagglutinins for platelets have been demonstrated in normal serum (635). The intensity of agglutination varies with the nature and quantity of the solution, the temperature and the animal species. Dog platelets are easily agglutinated, those of the cat and rabbit are less easily agglutinated than those of the dog and even less than those of man, sheep and donkey (25); donkey plasma, without anticoagulants, may coagulate while the platelets remain unagglutinated.

Starlinger and Sametnik (602) found that platelets in citrated plasma are negatively charged; dilution with physiological salt solution increases their charge greatly and progressively diminishes their cataphoretic velocity until the neutral point is reached and the platelets gather into clumps. According to Abramson (2), no change in the cataphoretic velocity of horse platelets in oxalated plasma occurs before or during their agglutination. Aggregation of platelets in plasma may occur without any measurable change in electrokinetic potential but may simply result from changes in adsorbed protein films causing an increased stickiness of the cells (2). In isotonic salt solutions platelets agglutinate at pH 3.5–4.5 (575) (298), in citrated plasma, pH 4.7–5.4 (602), thus falling within the range for the serum proteins (4). This may be an indication that platelets are surrounded by a firmly adsorbed protein film, as pointed out by Roskam (542), and that many of their properties are to be attributed to this film. When Novirudin, an anticoagulant, is injected into animals whose veins are subsequently injured, the platelets, although not showing any morphological changes, do not collect at the point of injury (12). This fact seems to indicate that the plasma contributes normally to the agglutination of platelets and that substances that prevent clotting check this property temporarily and those that accelerate clotting, also

accelerate agglutination. The platelets of patients with "constitutional thrombopathy" and cholemia are well agglutinated by normal plasma, while the plasma of these patients has no agglutinating effect on platelets isolated from the blood of normal individuals (457). Starlinger and Sametnik (602) have shown that the agglutination of platelets is notably influenced by the various changes in the blood observed in the course of certain morbid states and after parturition. The increase in proteins with a weak negative electric charge (fibrinogen, globulin) observed in these states, lowers the electric charge of platelets, increases their agglutinability, while mutual contact is further facilitated by a simultaneous increase in the number of platelets in the circulation.

The agglutinability of dog platelets is accelerated by protein intake while fats have no effect and carbohydrates cause a delay; platelet agglutinability is not related to increases in the basal metabolic rate of dogs brought about by feeding or thyroid extract; physical exercise (10 minutes duration) accelerates the agglutinability of platelets of dog and man; subcutaneous injection of tissue fibrinogen or of adrenalin accelerates it markedly (440).

According to Glanzmann (250), there is a delayed agglutinability and increased fragility ("thrombasthenia") in a type of hereditary hemorrhagic disorder, observed subsequently by several workers (447) (102) (413) (206) characterized by severe attacks of epistaxis and ecchymosis, a prolonged bleeding time and the absence of clot retraction. Glanzmann's demonstration of the nature of the defect has been confirmed by some workers (608) (337) and denied by others (343). Platelet agglutinability is lessened during experimental asphyxia (72) and in anaphylactoid purpura (298). There is also a decrease in hemophilic blood, a fact which has been suggested as the cause for the delayed blood coagulation in this disease (558) (308). Although the slow agglutination of platelets undoubtedly contributes to that delay, it does not seem to be its primary cause. Whatever is responsible for the increased stability of hemophilic plasma, would tend secondarily to render the platelets more stable (208). Platelet agglutinability has been found increased in diseases involving septic processes (689) after operative procedures (291) and in malaria (62). There may be

increased agglutinability simultaneously with the appearance of auto-agglutination of erythrocytes (689), thus suggesting a common cause for both phenomena.

Roskam (542) points out that the agglutination of platelets to each other and to foreign particles, their sticking to vascular lesions and the rôle they play in the formation of thrombi, are passive processes, mere expressions of changes in the colloidal equilibrium of the plasma when in contact with a foreign surface. The relationship between the colloidal equilibrium of plasma and the platelets must, however, be viewed as a reciprocal one; it is impossible to state, at a given time, which one plays the primary rôle. Alterations in the platelets themselves may affect the colloidal equilibrium of the plasma and vice versa. There is additional evidence, moreover, of the occasional dissociation between platelet agglutination and plasma coagulation. The injection of cocaine into dogs in amounts to produce a concentration of 2 grams percent, prevents or delays agglutination of platelets even after coagulation of the blood has taken place (174). Such platelets and the blood clots containing them, do not adhere to glass surfaces.

Physical alterations. Although agglutination of platelets is generally followed by fusion and lysis, it may be reversible, depending on the intensity and duration of the conditions bringing it about. The swelling and grouping of platelets into sticky masses, which gradually become granular, was described as the viscous metamorphosis of platelets by Bizzozero (76), who observed the change in the lumen of injured living vessels. The substances from the blood capable of producing this transformation are associated with the early stages of coagulation (683). The character and rate of the viscous metamorphosis of platelets from hemophilic blood does not differ from normal, after addition of substances causing that transformation in normal platelets (683). The transformation is best detected in serum and was first studied by Osler (484), who referred to the changed platelets as the "granular masses of Schultze." A leisured observation of platelets undergoing alterations is possible, if unfixed blood rapidly collected into citrate solutions is allowed to stand in hanging drop preparations, at room temperature. If the concentration of citrate is adjusted to allow these changes to occur, the platelets first lose their high refractility and take on a duller, more indistinct appearance.

Granules appear in the central portion, surrounded by a clear zone, which gradually increases in area. Such swollen forms are often seen sticking fast to the glass surface at the edge of the plasma. The little granular mass may be pushed to one side, the clear zone assuming a spherical outline. This spherical vesicular area remains sometimes attached to the granular mass for several minutes, then floats away and is lost in the surrounding fluid. On floating away the vesicles leave a group of deeply staining granules (Plate 1, Fig. 10-A) which may break free and show active Brownian movement. Some of these represent, perhaps, the "kinctocytes" described as increased in numbers in thrombopenic purpura (189). A few platelets do not undergo peripheral swelling but instead emit flagella-like processes, while the center shrinks and becomes more concentrated (Plate 1, Fig. 10-B). The processes may break off and float free in the fluid, occasionally attaching themselves to other cells. In normal mammalian plasma collected without precautions to prevent coagulation, agglutination precedes or accompanies these changes, which occur so rapidly that it is not possible to follow them in detail. In citrated platelet-rich plasma, platelets remain well preserved for several hours, then suddenly, and apparently simultaneously, all, whether singly or in clumps, begin to alter (Fig. 1, A). Ferguson (211) has pointed out that the surface tension lowering action of a wettable surface is the primary cause of platelet alteration. A final concentration of 0.25% of trisodium citrate is necessary to inhibit platelet lysis (rabbit blood) and this "critical concentration" is practically uninfluenced by the factor of osmotic tonicity (211). A process of "osmotic imbibition" is, however, necessary for the liberation from the platelet of the vesicular excrescences, and in this process the essential rôle is played by the calcium ions (210).

Separation of the platelet, in stained smears of blood, into two zones and the presence of insect-like forms (Plate 1, Fig. 8-A, B) result from the fact that most preparations are fixed and stained only after platelets have already undergone alterations. The vacuoles of stained platelets (Plate 1, Fig. 11) represent the vesicles formed during this transformation. The initial stage of these changes was taken as evidence of amoeboid motion by Deetjen (145) and Van Herwerden (647). The prolongations emitted by platelets do not retract,

however (326), and are not suppressed by phenol or chloroform which suspend the movements of leukocytes (25). Considering how easily platelets undergo alterations, once outside the body, any evidence of ameboid motion would have to be demonstrated "in vivo." Similar changes have been described by Tait and Gunn (614) for the thigmocytes of the crayfish. These cells have a round shape when examined in freshly collected blood but a few moments after standing, spinous processes sent out from their surface stretch over the glass slide. These processes are irretractile, thus differing from those sent out by amebocytes. Once stretched out over a surface, a thigmocyte cannot

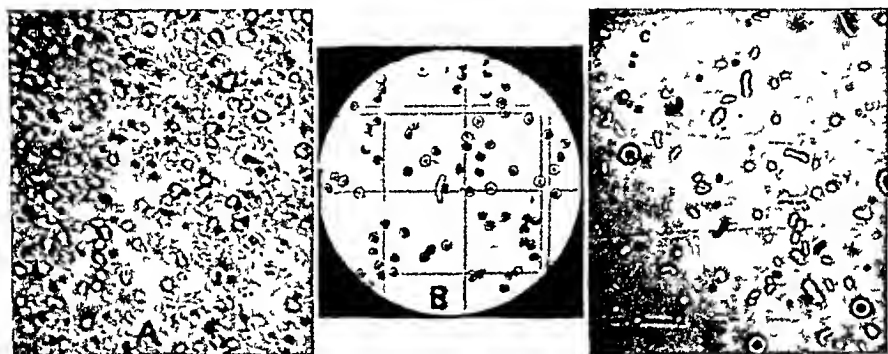


FIG 1. A Blood platelets from the dog, collected in 3% citrate solution and allowed to stand in a moist chamber for 2 hours. Nearly all platelets have become swollen and granular. $\times 336$ B. Giant banana-shaped platelet from the blood of a dog recovering from experimental thrombopenic purpura. Suspended in citrate formalin solution $\times 182$. C Abnormal forms from the blood of a patient with chronic thrombopenic purpura. Suspended in citrate formalin solution. $\times 336$.

displace itself any more; its behavior is, therefore, conditioned solely and directly by capillary forces and surface tension. The expansion of platelets coming into contact with "wetable" surfaces is compared by Tait (613) to the progressive increase in area of a drop of water when placed on a stone. When expansion goes beyond a certain limit, cytolysis takes place. Disintegration of these bodies appears to be simply the result of overstretching, which may reach dimensions of 100 square micra for large and 40 square micra for small platelets (614). Tait and Gunn (614) demonstrated that, with the hyalin thigmocytes of *Astacus*, cytolysis is immediately followed by coagulation of the surrounding plasma. Many of the spindle cells of amphi-

ans and fishes, certain spinous cells of the blood of *Astacus* and other crustacea, various corpuscles of mollusca, arthropoda and annulata behave, in this respect, as the mammalian platelet (613).

The rate of disintegration of platelets has been measured in various ways. In urea (10%) citrate solutions, 60–70% of the platelets normally disintegrate in the first 20–40 minutes, after which the rate of disintegration is minimal (32). In citrate and metaphosphate solutions (32) (481), the rate of disintegration is slower. In 0.5% citrated blood, platelets from man and rabbit, respectively, disintegrate at the following rates: 12 and 16% in the first hour; 31 and 28% at the end of 3 hours; 59 and 62% at 24 hours (353). In citrate solutions, regardless of their concentration, only 10–20% of platelets from cutaneous or venous blood are destroyed in the first 20 minutes and 20–40% in the first 40 minutes. Platelets from thrombopenic individuals are slower in disintegrating, which may indicate that younger platelets are more resistant than the older forms; hemophilic platelets do not behave differently from normal (32). Although valuable information has been obtained by these methods, it would seem that the resistance of platelets to disintegration should be tested in solutions of tissue juices, which behave towards platelets as veritable antibodies (25).

Chemical composition.—It is difficult to obtain platelets entirely free of plasma for chemical analysis. According to Aynaud (31), the washed platelets from the horse and donkey give the usual protein reactions, fifteen percent of their dry weight is soluble in alcohol, ether or chloroform, and the ash contains phosphorus, iron, sulphur and calcium. Alcohol extracts of platelets, when evaporated, yield no crystals but irregular masses of fatty appearance and rancid odor. Platelets from the horse yield from 4% to 5.7% of their dry weight in ash (31) (278) (194). The composition of horse platelets is: protein 71%, lipoid 12%, cholesterol 1.7%, ash 5.5%, calcium 0.074% (278); in one hundred cubic centimeters of a tightly packed mass, the following elements are found (194): sodium: 272.–300. mg.; potassium: 50.–81.5 mg.; calcium: 6.1–11.0 mg.; iron: 0.51–0.91 mg.; chlorides: 238.–287. mg.; inorg. P.: 24.3–29.1 mg.; and bicarbonate: 9 volumes percent. In two batches of horse platelets Chargaff, Bancroft and Stanley-Brown (125) found an average of 12.9% lipids (dry weight). The acetone insoluble lipids consisted of phosphatides from which

lecithin and cephalin were obtained; the acetone soluble fraction consisted mostly of free and esterified cholesterol (125). There is a close similarity between the composition of platelets and leukocytes of horse blood, the main differences being in content of water and salts. Both differ markedly in their mineral content from plasma and erythrocytes (194). In cation concentration horse platelets and leukocytes occupy a position between plasma and erythrocytes; they contain less sodium and calcium but more potassium than plasma. With respect to anions, the chloride concentration of horse platelets and leukocytes is about the same, standing between that of the plasma and erythrocytes; they contain, however, more inorganic phosphorus and less bicarbonate than plasma and erythrocytes. Horse platelets have more sodium and calcium than leukocytes, but less potassium and iron; their calcium concentration is approximately like that of the plasma but contain three times as much iron as the latter (194). In man the lipid distribution of platelets indicates a composition similar to that of erythrocyte stroma. In 34 samples examined by Erickson, Lee and Williams (199a), the lipid distribution expressed as per cent of dried platelets was: total lipid 15.4, phospholipid 11.4, free cholesterol 2.5, cholesterol esters 0.9, neutral fat 0.6, and protein 64.0. Sixty-eight per cent of the phospholipids consists of cephalin. The platelets from hemophilic subjects have a lower phospholipid fraction and a higher proportion of neutral fat than those from normal men, though the percentage of phospholipid present as cephalin in hemophilic platelets appears to be normal (199a).

Besides evidence regarding the composition of platelets obtained by direct analysis, insight into their chemical nature has been obtained by noting their response to various staining reagents. The granules of platelets show different tinctorial reactions from those of leukocytes. Using the triacid reagent of Ehrlich, Aynaud (25) could not demonstrate any neutrophile, basophile or acidophile granules in mammalian platelets. Platelets have little affinity for cytoplasmic stains, such as eosin or orange iodo-eosin. Aqueous solutions of methylene blue, Nile blue, toluidin and thionin stain fixed platelets a very pale blue, much paler than those of the protoplasm and nucleus of leukocytes. Pyronin, a basic dye which stains red the protoplasm of mononuclears, does not stain platelets, a fact which led Aynaud (25) to question the

likelihood of platelets originating from the cytoplasm of those cells. Nuclear dyes (methyl green, hematein) stain platelets weakly and do not demonstrate a nucleus in them. The platelets of most mammals show a strong affinity for the Giemsa stain mixture. Contrary to the leukocytic granulations, it is possible to demonstrate the Giemsa stained granules of platelets by many methods of fixation, even after treatment with normal HCl (131a) and for a long time after the blood has been outside the body. This granular substance is dissolved in the hyaline material when the platelet is well preserved but precipitates out at the least alteration provoked by conditions in the surrounding medium. The relatively high quantity of substances soluble in lipid solvents, the presence of phosphorus, the staining reactions of platelets, their insolubility in gastric juices and digestion by pancreatic juices seemed to Aynaud (31) to constitute arguments favorable to the existence in platelets of nuclear substances in a diffuse state. According to one of the first chemical analyses of platelets (410) these corpuscles were made up of a combination of nuclein and albumin.

Platelets yield a negative peroxidase reaction (532) (259) but contain an oxidase (342) (315). According to Roskin and Grunbaum (550), platelets yield a positive dopa-oxidase reaction and negative reactions for other oxidases. Like leukocytes, platelets contain a catalase (316) which, like the oxidase, is a function of its granules. Since only leukocytes of myeloid origin contain a catalase (315), this constitutes indirect evidence of the myeloid origin of platelets (316). No proteolytic or lipolytic enzymes have been demonstrated in dog or rabbit platelets (544); horse platelets have a strong peptolytic enzyme which splits various polypeptides and, in particular, glycyl-l-tyrosine (1).

Respiration. The first indication that platelets respired oxygen was the demonstration by Aynaud (25) that fresh suspensions of platelets decolorize dilute solutions of methylene blue. Platelets consume appreciable amounts of oxygen (656) (482) (415), the consumption being increased by shaking the platelets with glass beads, diminished by simple centrifugation of the plasma (482), and unaltered by standing for 24 hours at 5°C (193). The oxygen consumption per hour, per mg. of human platelets, is 8.4 mm.³, of dog platelets, 5.0 mm.³ (193).

Electrical properties. The electrokinetic potential of platelets of man lies between 20 and 42 millivolts, the potential being in 50% of the cases between 25 and 30 millivolts (138). There are variations in the potential at various times of the day but no clear relationship may be established with any definite time or with food ingestion (138). In oxalated plasma, horse blood platelets, whether singly or in clumps, have an electrokinetic potential of 12 millivolts which remains unchanged for several hours after collection of the blood (2). The cataphoretic velocity of horse platelets in oxalated plasma is 0.45μ (range 0.4–0.51) per second, per volt, per cm. Horse platelets migrate in the electric field 90 percent more slowly than erythrocytes, 15 to 30 percent more slowly than lymphocytes and at about the same speed as polymorphonuclear leukocytes (2). This indicates that the surface of platelets is similar to that of polymorphonuclear leukocytes but should not be interpreted as indicating a similar origin of the two elements, for quartz particles in serum migrate with the same velocity as leukocytes (3). The surface similarity of platelets and polymorphonuclear leukocytes is probably due to similarities between the proteins adsorbed to the two corpuscles. Further indication of this probability is the fact that many of the bacteria that stick to platelets also stick to leukocytes and those that do not stick to one do not stick to the other (254). The cataphoretic velocity of platelets from an immunized rabbit, or of normal rabbit platelets placed in contact with an immune serum is less than that of normal platelets in normal serum (593). Platelets are negatively charged whether in citrated plasma (602) or in a solution of 0.3% NaCl and 5% sucrose (257). In the latter solution *B. coli* are also negatively charged, so that the agglutination of platelets to these organisms is not due to the attraction of two particles of opposite sign. According to Stuber and Lang (608), platelets from individuals with hemophilia and "thrombasthenic purpura" have higher than normal electric charges and their isoelectric point is found at lower molar concentrations than normal platelets.

The comparative electrical conductivity of horse blood corpuscles in plasma is: platelets = 70; erythrocytes = 100; leukocytes = 81. Freezing and thawing, standing in the ice box (5°C, 24 hours) or

saponin (0.2%) do not change the electrical conductivity of platelet suspensions (195).

Pharmacological action. Significant information regarding the composition and function of platelets has been obtained by comparing the pharmacological action of platelet rich plasma with that of the same plasma after the platelets have been removed by centrifugation, and of serums obtained from platelet-free and platelet-rich clots. O'Connor (474) and Stewart (607) showed that while serum has a marked vasoconstricting action, plasma itself has little. Breakdown of blood platelets is accompanied by liberation of a vasoconstrictor substance (322). The vasoconstrictor property of shed blood is directly proportional to the number of platelets in the circulation; defibrinated blood from patients with a low platelet count has much less vasoconstrictive effect than that from individuals with a high platelet count (294). On isolated arterial rings, or frogs' hearts, aqueous extracts of rabbit and horse platelets exert a strong constrictive action (607) (406) (474) (617), which is more marked with aging of the platelet suspensions (357). The vasoconstrictive power of normal serum and platelet extracts may result from a stimulating effect on smooth muscle of substances liberated from the platelet during its disintegration. Analogous extracts of spleen, thymus and bone marrow do not show a similar vasoconstrictive action (691). According to Roskam (546), the active principle is dialysable while Tanaka (617) finds it incapable of being dialysed. The principle is thermostable (546) (617), can be totally adsorbed on animal charcoal (617), easily dissolved in alcohol, less in ether or chloroform (546) (617), and appears to be an amine in a colloidal state (617).

In spite of their marked vasoconstrictive action on isolated vessel rings, aqueous extracts of rabbit platelets, or rabbit serum derived from platelet-rich plasma, when injected intravenously, lower the arterial blood pressure of the rabbit and dog (404) (406) (545). The hypotensive principle is thermostable (546) and is not present in aqueous extracts of dog and human platelets (545). The hypotensive action of the extract seems to be due to the dilatation that it produces in the capillaries of the kidneys and extremities even though it has a constricting action on the spleen and large vessels (546). Thus the

vasomotor action of platelet extracts resembles that of histamine. The fall in blood pressure that follows injection of platelet extracts is analogous to that observed after injection of various organ extracts (96) and is due to a predominance of the vasodilating effect over the vasoconstricting one (546).

An increase in the tone and amplitude of peristaltic contractions of the small intestine follows direct local application or intravenous injection of the products of platelet disintegration or extraction (357) (617) (235). The intestinal peristalsis of dogs during the acute stage of experimental thrombopenic purpura brought about by destruction of platelets with an anti-serum, is markedly accentuated (634). Freund (234) and Klecki and Pelczar (357) drew attention to the fact that a rise in body temperature follows injection into the circulation of the products of platelet disintegration. In thrombopenic animals and in some human beings with chronic thrombopenia of several years standing, moderate elevations of the body temperature (1° – 2° C) may be observed regularly (634). The rise in temperature that follows injection into rabbits of defibrinated blood (234) and that occasionally follows blood transfusions in man is due in a large part to products of platelet disintegration.

THE PRODUCTION OF PLATELETS

Origin. Much controversy has centered about the origin of blood platelets. A consideration of the most important theories is worth while for the light it casts indirectly on various aspects of this problem. The principal views may be resumed as follows:

1. Platelets originate as a precipitate from the plasma when the blood is shed and do not, as such, exist in the circulation (679) (103) (427) (93). If blood is collected from the carotid artery of a rabbit directly into absolute alcohol, no platelets appear in the mixture; the more water is added to the alcohol, the more platelets may be found (427). The same is true if sublimate or osmic acid are used as fixatives, which to Marino (427) was an indication that the blood of an animal would or would not contain platelets, depending on the medium employed for collection. This is obviously no reason to deny the existence of platelets, for the addition of absolute alcohol is certain to alter profoundly the physical state of the blood. Considering the

precautions that must be taken to preserve platelets for demonstration outside the vessels, it is natural that such a view as this should, at some time, have been advanced. Furthermore, the demonstration of the presence of platelets in the circulating blood of vessels of living animals has been so repeatedly made that their existence as formed elements in normal blood is no more open to question than that of erythrocytes.

2. Platelets originate from the endothelial lining of blood vessels. Support for this hypothesis, towards which have leaned several workers (459) (398) (25), rests chiefly on Lowit's (417) finding that stasis, produced artificially in a vessel, seemed to increase the platelet content in that vessel, a fact that has been disputed by Preisich and Heim (516). Recent studies (634) have shown that there is, under certain conditions, a greater number of platelets in venous than in the arterial blood. This may be a result of temporary sequestration and storage of platelets in capillaries having a stagnant or nearly stagnant circulation. Once the circulation is reestablished, these platelets are thrown back into the blood current and pass through the veins. At this particular time the platelet content of venous blood may exceed that of arterial. This supplies a partial explanation of the increase in platelets in the venous limb of the circulation after a period of artificial stasis although it obviously does not exclude the possibility that they may originate from the endothelium of capillaries and venules in portions of the body other than those observed. The evidence in favor of this view is, however, far from satisfying. The fact that after a period of thrombopenia, induced either naturally or experimentally, the increase in the platelets is usually detected first on arterial blood (629) argues against the theory.

3. Platelets originate from blood cells.

(a) From erythrocytes. This view originally upheld by Weidenreich (662), Neumann (468), Mosso (460), Engel (196), Arnold (20), Fr. Muller (463), Maximow (429), Hirschfeld (296), Petrone (500), Schwalbe (578), Spadaro (594), and Gruner (268) was revived recently by Watson (658). Most of the evidence in support of this theory rests on similarities, tinctorial and morphological, between platelets and certain portions of altered erythrocytes. The presumable increase in the platelet content of shed blood, also brought forth as an

argument in favor of this view, can be accepted only by those who designate as platelets all sorts of products of the disintegration of leukocytes, erythrocytes and of the platelets themselves. It is easy to confuse any cellular fragment or particle with a platelet since many of the morphological qualities of these corpuscles are also common to other microscopic bodies. Adhesion of altered platelets to erythrocytes, further complicates the problem. The bodies liberated by erythrocytes during hemolysis are not platelets. There is no numerical increase in blood platelets after injections of hemolytic or anti-erythrocyte sera; the fragments extruded from red blood cells do not undergo clumping and fusion as do platelets, and are, moreover, inert as clot-retraction-inducing agents. Altogether, the evidence for this origin of platelets is indirect and inconclusive. Scant support is likewise available for the opinion of some authors (197) (321) (263) (569) that the platelet originates from the nucleus of normoblasts. According to one investigator (568), the erythrocyte normally retains its nucleus, but when subject to unusual conditions, the nucleus with a small amount of cytoplasm is liberated as a platelet.

(b) From leukocytes. The claims in support of this view rest chiefly on the form, tinctorial characteristics and specific gravity of platelets, and on their content of nucleo-albumin (410). Wernicki (672) attributed the origin of platelets to the granulations of eosinophiles and Marino (427) to the nuclei of degenerating white corpuscles. Riess (534), Schwalbe (579), and Grawitz (263) were among the supporters of this theory, which has been attacked by Pappenheim (491), Weidenreich (662), Aynaud (25), and Downey (169). The latter two have pointed out that platelets stain weakly with basic and acid dyes, and quite differently from the nuclei of leukocytes. The affinity of platelets for the Giemsa stain does not necessarily relate them to nuclear material, for that affinity is more of the nature shown by protozoan than by mammalian nuclei (25). Platelets do not stain with methyl green, a dye regarded by Pappenheim (491) as being specific for metazoan chromatin. Moreover, an array of facts militates against this view: platelets are numerous in fetal blood when leukocytes are extremely rare (25); there is no significant correlation between the number of leukocytes and platelets in the circulating blood; injections of antileukocyte serum affect the

number of platelets but little, and antiplatelet serum has, likewise, little effect on the number of leukocytes (52); leukocytes do not induce retraction of the clot, a property characteristic of platelets (622); platelets are absent from many areas where leukocytes are numerous, as in the lymph, peritoneal transudates, aqueous humor, and abscesses (25). The fact that platelets occasionally show parallel changes to the leukocytes in the leukemias and leukopenias, may only indicate that any process affecting leukocytes may by continuity or contiguity affect platelet regenerating tissues, or the platelets themselves, an explanation that may also apply with respect to pathological changes in the erythrocytes and erythropoietic tissues.

(c) By direct multiplication or differentiation from the platelets themselves (594). This theory is based chiefly on the appearance of mitotic figures in platelets (496). The varied aspects presented by platelets undergoing alterations and the fact that they are deprived of nucleus, make it unlikely that the appearances described as mitotic figures are such indeed. Nucleated platelet forms have been described various times and the view advanced that they may represent the precursors of the normal platelet (53), which is thus endowed with an independent genetic line similar to that of the erythrocyte (145) (148). The evidence in favor of this view is extremely scanty (55). Even in very young guinea pig and rabbit embryos, no truly nucleated platelet forms may be found. It is more likely that nucleated erythrocytes have been taken for such forms.

4. Platelets arise from the hematopoietic organs.

(a) In the lymph and hemolymph glands. This view has been upheld principally by Czermack (140), Dominici (166), and, recently, was partially adopted by Spadolini (595). It rests mainly on similarities in the staining properties of platelets and the pseudopods of the cytoplasm of cells of lymph glands, though Aynaud (25) and Foa (226) were unable to confirm such similarities. When Donn  (167) first described his "globulin" he evidently assumed that the hyalin bodies found in the lymph and platelets were one and the same thing for he gave the lymph as the origin of the "globulins." Downey (169) showed by a careful fixing and staining technique that the hyalin bodies constricted off from the cytoplasm of lymphocytes and mononuclears in the rabbit are in no way related to blood platelets. He

followed the bodies from the lymph glands to the lymph vessels, into the thoracic duct and, finally, into the circulation, and showed that they disappear rapidly in the thoracic duct, very few of them ever reaching the general circulation. It is not unlikely, however, that in the leukemias, for example, these bodies reach the circulation in greater numbers. The fragmentation of platelet-like bodies from leukocytes may be observed sometimes in the blood (108). A serious objection to this view is the fact that of antisera prepared against various tissues (spleen, bone marrow, lymph glands, endothelial leukocytes) only anti-bone marrow and anti-spleen sera cause a thrombopenia (55). The views of Spadolini are a continuation and amplification of those first suggested by Dominici. While the latter held that platelets arise from cells of the lymphoid follicles of lymph nodes, Spadolini (596) attributes their origin to cytoplasmic buds from cells of the reticulo-endothelial system of the pulp. Although admitting that platelets may be derived from megakaryocytes, Spadolini considers this mode of origin as unimportant, that from the reticulo-endothelial cells being the rule.

(b) In the spleen. The spleen is the only organ in the parenchyma of which platelets may be definitely demonstrated. They are often found in masses or rows along the sinuses or in medullary cords, but not in the follicles. Foa and Carbone (224), Aschoff (24), Pappenheim (492), Aynaud (25), LeSourd and Pagniez (407) and Cesaris Demel (124) believe that at least some of the platelets originate in the spleen. This organ, however, cannot be the only or chief source of their formation, for splenectomy is nearly always followed by an increase in the number of platelets in the circulation.

(c) In the bone marrow. It is believed today by most authorities that platelets originate from the megakaryocytes of the bone marrow. J. H. Wright (680) pointed out this mode of origin in the cat and his work was later confirmed by Ogata (477) and others (403) (169) (466) (212) (245) (346) in the rat, guinea pig, rabbit, cat and man. Wright's conception of the origin of platelets may be told best in his own words: "All of the blood platelets are detached portions or fragments of the cytoplasm of the megakaryocytes, which are in such relation to the blood channels in the marrow that detached portions of their cytoplasm are quickly carried by the blood current into the circulation.

The breaking up of the cytoplasm into the platelets occurs only in cells which have reached a certain stage of growth and development, and is probably rapidly completed when once begun. It takes place in various ways but usually by the pinching off of small rounded projections or pseudopods from the cell body or from larger pseudopods, or by the segmentation of slender pseudopods, or by the pinching off of longer or shorter pseudopods which may or may not undergo segmentation later. All or most of the cytoplasm of the giant cell is given off to the blood stream and the nucleus degenerates. The more or less naked nucleus is often carried by the blood stream to the lungs where it lodges in the capillaries" (681). To account for the absence of platelets in birds and lower vertebrates, Wright advanced the view that in these animals the thrombocyte plays the rôle of the platelet but is analogous morphologically to the megakaryocyte of mammals. There are no megakaryocytes in the bone marrow of birds; the giant cells circulate in the blood stream as thrombocytes (681).

The problem of the origin of platelets has been made the object of intensive study and most of the evidence so laboriously accumulated is, when critically analyzed (124), impressively in favor of Wright's theory. The dissenters present arguments which are either polemical in character (496) or do little to invalidate the theory (503). Gruner (268) raises objections to Wright's theory on the ground that 1) the large quantity of platelets in the blood would require an enormous quantity of megakaryocytes that could not be found in the bone marrow of any animal; 2) platelets may be found in Crustacea, animals without a bone marrow and, therefore, without megakaryocytes. Both of these objections, however, may be overruled by the view recently proposed by Howell (310), that the greatest production of platelets from megakaryocytes takes place in the lungs. Brown (97) pointed out that the granules characteristic of platelets and megakaryocytes may be found in other tissue cells and in transitional leukocytes, which under conditions of excessive demand for platelets, may take part in their production by segmentation of the cytoplasm. For reasons already given (vide p. 176) care must be taken not to confuse any and all cellular fragments with platelets. Petri (499) undertook an extensive investigation of Wright's theory. He ob-

served the effect on blood platelets and the number and types of megakaryocytes of the bone marrow of rabbits, of the injection of various substances (peptone, saponin, pyrocin), of single, large hemorrhages, repeated small hemorrhages, asphyxia, and the "ideal platelet bleeding" (repeated fractional centrifugation of large amounts of citrated blood, removal of the platelets, reinjection of the citrated blood into the animal). Besides the fact that this method of producing a thrombopenia is not practical, for most of Petri's animals died during the period of the experiments, it is questionable whether it may be called "ideal," involving, as it does, the injection of large amounts of citrate. Though Petri's experiments yielded negative results, they do not, of course, disprove the validity of Wright's theory, for evidence in its favor is available in many ways. Petri's experiments seem to have shown merely that in rabbits (animals poorly suited for this demonstration) and by his own methods, the adequacy of which is doubtful (124), the investigator himself was unable to find any relationship between platelets and megakaryocytes. On the other hand, Gunn (271) found a certain relationship between qualitative changes in the giant cells and the number of circulating platelets in rabbits, even though he was unable to find a positive correlation between the number of platelets in the blood and the number of megakaryocytes in the marrow.

The majority of hematologists consider the origin of platelets from megakaryocytes a settled question (213) (226) (24) (232). Experimentally, Wright's theory was upheld by Bunting (106) (108), who observed an increase in the number of megakaryocytes and in the fragments issuing from their pseudopods coincident with the rise in the number of blood platelets induced by repeated bleeding or the injection of hemolytic substances. Similar relationships have been demonstrated after the thrombopenia produced by injections of benzol (668), antiplatelet serum (55) (633), lead and pyrocin (212) (157), and saponin (217) (203) in rabbits, guinea pigs and dogs. Blocking of the reticulo-endothelial system in mice is followed by a thrombocytosis, the appearance of megakaryocytes in the peripheral blood, and a simultaneous increase in their number in the spleen, and in their thrombopoietic activity in the bone marrow (650). Fragmentation of the cytoplasm of megakaryocytes giving rise to platelets

has been observed in fresh preparations of bone marrow (55) and of leukemic blood (556). Actual formation of platelets from megakaryocytes in the peripheral blood has been noted in various pathological conditions, particularly in the leukemias (466) (120) (226) (156). Other facts lend further support to the mode of origin proposed by Wright. Platelets begin to be observed in the embryo at about the same time mature megakaryocytes appear (466). There is a close similarity between the chemical composition of the cytoplasm of megakaryocytes and of platelets (131a). The granules of megakaryocytes and of platelets from vessels and blood smears, as brought out by the oxidase reaction, have the same size, form and stain (343). Rieckenberg's reaction may be demonstrated with the megakaryocytes of the bone marrow but not with the leukocytes (269); it may also be demonstrated with the spindle cells of birds (269), thus supplying another argument for the common origin and functional analogy between thrombocytes and mammalian platelets. There are only two tissues that, when added to blood which yields irretractile clots, will induce retractility: the splenic pulp and the bone marrow, the former because of its richness in platelets, and the latter because of its megakaryocytes. If a suspension of splenic pulp is allowed to sediment and to clot in a glass vessel, retraction will appear in the upper layers of the pulp, that is, where the platelets are located. If a bone marrow pulp suspension is allowed to sediment and clot, retraction takes place in the lower layers of the clot, where the heavy megakaryocytes may be found (403).

Distribution of megakaryocytes. Megakaryocytes are always present in normal adult mammalian bone marrow. In the fetus they appear in regular sequence, first in the liver, and subsequently in the spleen and marrow (310). In the spleen of adult animals they are found in greatest number in the pig and, in order of highest frequency, in the cat, dog, rabbit, guinea pig, goat and lamb (503). In the liver they are rarely found normally in adult animals, but may still be present just before birth, in kittens, rabbits and guinea pigs (503) and, shortly after birth, in cats (310) and dogs (633). They are rarely found in the lymph glands and never in the omentum (503). Megakaryocytes begin appearing in the lung of the cat (310) shortly after birth. In normal mammalian lung, typical megakaryocytes are not easily

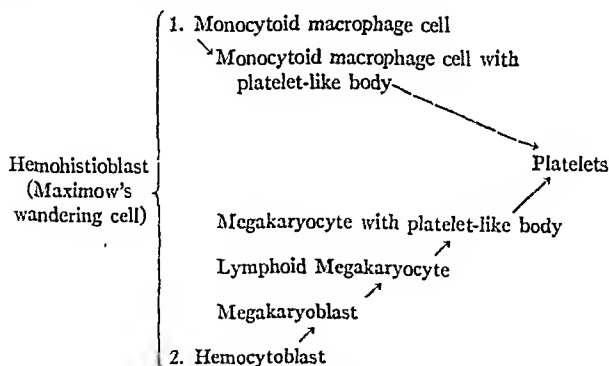
demonstrated; only the nucleus with very little cytoplasm may be noted. Increased numbers of these cells appear in the lungs a few days after spontaneous (23) or experimental fractures (503), blood defibrination (310), or a thrombopenia induced by injections of saponin (106) (310), antiplatelet serum (633), or peptone (310).

The giant cells are usually located near or in the blood vessels, especially the veins (551), of the spleen, bone marrow and liver (681) (121). Howell (304) pointed out that the homogeneous substance surrounding the cytoplasm of megakaryocytes spreads away from the body of the cell towards the lumina of vessels. The location of megakaryocytes near veins, where there is apt to be a greater lack of oxygen than in other parts of the circulation, suggests that the stimulus for their development is probably linked with varying degrees of oxygen deficiency (551). This is rendered more likely by the fact that asphyxia in kittens is accompanied by a thrombocytosis and a sudden increase in the number and activity of the megakaryocytes of the spleen (122).

Origin of megakaryocytes. According to most authors, there are two types of giant cells in normal bone marrow: 1) the polykaryocytes (304), (osteoclasts of Kolliker) (362), which have multiple, separate and similar nuclei; and 2) the megakaryocytes (304), (giant cells with budding nucleus, of Bizzozzero), characterized by a single large multilobed nucleus. Howell (304) considered the two cells as entirely distinct from each other and probably having different functions, the polykaryocytes being formed by fusion of a number of smaller cells. Gandolfo (242) has pointed out that polykaryocytes are not osteoclasts for they are normally found in organs like the spleen and liver which do not have any osseous tissue. There are two principal views concerning the origin of megakaryocytes:

1) Megakaryocytes are derived from hypertrophy of the cytoplasm and multiplication of the nucleus of a primitive undifferentiated polyblastic cell (554) (212) (213) (327) (170). From this cell originates the hemocytoblast (the primitive, indifferent blood cell), and from the latter is differentiated a large element with a non-granular, intensely basophilic cytoplasm and a large single nucleus, either oval or kidney shaped, provided with numerous nucleoli (212). These elements which are designated megakaryoblasts (Plate 1, Fig. 1),

specific progenitors of the megakaryocyte, may originate in a few instances directly from the lymphoid undifferentiated polyblastic cell (Maximow's wandering cell or Ferrata's hemohistioblast). From the megakaryoblast is developed the "lymphoid megakaryocyte," a large cell with basophilic protoplasm without granules, and with a monomorphous or polymorphous nucleus (Plate 1, Fig. 2). Finally, this cell differentiates into the mature megakaryocyte the cytoplasm of which is full of the characteristic azurophilic granules grouped at first in the perinuclear zone (Plate 1, Fig. 3) and later spread over the entire cytoplasm (Plate 1, Fig. 4). It is only when the cell is at this stage that it is capable of producing platelets. When production is stepped up owing to an increased demand for platelets, segmentation may take place directly from the megakaryoblasts, thus giving rise to abnormal platelet forms with coarse granules spreading evenly over the entire surface of the pseudopod-like mass.



Scheme of Origin of Platelets and Megakaryocytes. (Ferrata and Negreiros-Rinaldi) (212)

In the embryo, platelets may originate through either or both of the above processes, in the normal adult through the 2d process only (212). In the relatively young, human embryo (10 to 12 cm. in length) with active hepatic hematopoiesis, megakaryoblasts, lymphoid megakaryocytes, and megakaryocytes with azurophilic granules may be found. During the prehepatic hematopoietic period, when there are no plate-

lets in the blood, all megakaryocytes in the embryonal mesoblast are either in the megakaryoblast or lymphoid megakaryocyte stage and, therefore, incapable of producing platelets (212).

2) Megakaryocytes originate by fusion of other cells, the polykaryocytes. Based on experimental and clinicopathological observations, Di Guglielmo (159) advanced the view that polykaryocytes and megakaryocytes represent different stages of development of the same cell. The mature megakaryocyte is derived from a primitive histioid cell with a basophile cytoplasm and a single nucleus, the "prepolykaryocyte." These cells have a tendency to grouping, with formation of mosaic like figures and cellular syncytia. Grouping of the cells is followed by fusion of adjacent cytoplasm and formation of the "polykaryocyte," a large cell with multiple, separate nuclei. These nuclei fuse with each other and thus constitute the megakaryocyte which may be more or less mature, depending on the shape of the nucleus and staining of the cytoplasm. The megakaryocytes would, therefore, originate according to the following scheme: Prepolykaryocyte \rightarrow polykaryocyte \rightarrow young megakaryocyte \rightarrow mature megakaryocyte.

The prepolykaryocyte of Di Guglielmo is a mononuclear cell exhibiting a marked tendency to confluence and fusion with other cells, with abundant, vacuolated, basophilic cytoplasm, free of granules, and a clear, reticulated nucleus with one or more nucleoli. (For figures illustrating these various cells and intervening stages, consult Di Guglielmo (159), Morone (458), and Torrioli and Scalfi (638). This mode of formation of megakaryocytes has been confirmed by Cesa-Bianchi (117) in a case of myeloid splenomegaly, and by others in the spleen of cats that have had repeated injections of antiplatelet serum (73), in the embryonal bone marrow, spleen and liver of various mammals and in the hematopoietic organs of adult animals treated with saponin, pyrocin and lead (242), and in the bone marrow of patients with Paget's disease of the bone (458). The view that megakaryocytes resulted from the fusion of single nuclear cells, one time advanced by Blumenthal (82), was recently strengthened by observations on saponin treated rabbits (433), and in tissue cultures of megakaryocytes (638). Lambin and Lamers (380) criticize Di Guglielmo's conclusions and point out that the latter's prepolykaryocytes are really

osteoblasts although Morone (458) clearly differentiates between osteoblasts and prepolykaryocytes. Lambin and Lamers (380) stress the fact that prepolykaryocytes are usually found almost exclusively in the zone of bone marrow active in bone formation, that is, near the costal cartilages, a fact interpreted by Di Guglielmo (161) as due to the greater hematopoietic activity in that zone. Transitional forms between the multinucleated (polykaryocyte) and the polymorphonucleated (megakaryocyte) giant cells had, at one time, been mentioned by Arnold (19) who considered them as different stages of the development of the same cell, which at first was uninucleated and then divided into many nuclei.

From the size of megakaryocytes, one would think that, if they were formed by division of the nucleus, transition forms between the original cell and the adult megakaryocyte would be found; yet such forms are rare (433) (203). The process of development of megakaryocytes is seldom accompanied by karyokinesis. A study of megakaryocytes of the bone marrow, spleen, liver and lymph nodes of rabbits and dogs treated with saponin, led Fabris (203) to propose what amounts to a harmonizing of Ferrata's and Di Guglielmo's views. The mature megakaryocytes originate in 2 ways: from the confluence of several distinct cells (megakaryoblasts) or from the successive and incomplete budding of the single nucleus of one megakaryoblast. It is probable that sometimes both processes may take part in the formation of one giant cell. The rosette or conglomerate nucleus type of megakaryocyte appears to be related more with origin by fusion (633) and the globular, vesicular nucleus type to proliferation of a single nucleus. Moreover, one must be guarded against depending too much on nuclear structure as a criterion of the age of these giant cells and their mode of origin. Practically every conceivable kind of nucleus can be obtained by slicing a single nucleus of a giant cell in different planes (344). The quality of the cytoplasm and of the granules gives a better indication of the age of the cell.

Sites of platelet production. Platelet production may occur wherever mature megakaryocytes exist: bone marrow, spleen, liver, lung. Most students of the subject regard the bone marrow megakaryocytes as the principal source of this production. There are a few facts, however, which seem to throw some doubt on this view and to point to the

lungs as an important if not the chief site for this formation (310). In tissue sections or smear preparations, fewer platelets are found in the marrow than in the spleen or lung. This may be due partly to the fact that the marrow may clot before being spread or fixed, the platelets undergoing disintegration. Repeated injections of anti-platelet serum into kittens eliminate the megakaryocytes from the spleen, though many may still be found in the lung (73). Perfusion of marrow yields few platelets (171) (310), while lung perfusates are rich in them (310). While this fact may be interpreted as indicating the presence of a large supply of platelets in the lungs, it is not entirely conclusive, for perfusion of the spleen, where platelets abound, is not followed by similar results (310). Though attempts to correlate fluctuations in the number of blood platelets with the number of marrow megakaryocytes have failed, such failure must be attributed in part to the difficulty of obtaining truly representative samples of marrow. The fact that the number of platelets in the peripheral blood is usually higher in the arteries than in the veins is susceptible of two interpretations: (a) platelets are added to the blood in the pulmonary circuit; (b) platelets are used up as the blood goes through the capillaries. Acceptance of the views of Howell and Donahue (310) must await further investigation of the problem. Unpublished experiments of Tocantins and Bradshaw on live cats with the heart and great vessels exposed by Drinker's (173) method, show an inconstant relationship between the number of platelets in the pulmonary artery and in the vein.

Mechanism of production. The circumstances surrounding the mechanism of separation of the platelets have been studied by Cesaris Demel (123) and by Betances (67). Both authors concluded that the separation does not depend on the penetration and fragmentation of protoplasmic prolongations of these cells in the lumina of blood vessels, but on a more complex mechanism. The cell plays a purely passive rôle (123). The platelets do not detach themselves until the cell begins to increase in size and the nucleus to divide amitotically, or, in other words, when the cell begins to degenerate. At this point the current of the blood detaches the platelets from the cells located near the sinuses or in their lumina. According to Cesaris Demel (123) platelets are derived directly from the blood plasma by a process of

precipitation conditioned specifically by certain elements present in the hematopoietic organs (premegakaryocytes) which are the physiological stimulants for this process. This stimulus determines the precipitation from the blood plasma, with which the premegakaryocytes are in direct contact, of a substance which adheres to these cells and increases their volume, thus giving them the characteristics by which they are known. In living tissues this mass is very fine and tenuous, semi-fluid, and under the influence of the blood current it becomes deformed, stretched and broken up into fragments which, under normal conditions, are of approximately the same size, but under pathological conditions may be of various sizes and shapes. The granular mass surrounding the megakaryocyte cannot be strictly considered as its cytoplasm (123). It has indistinct contours, it is easily deformable, varies widely in quantity and has the characteristics of a precipitation mass from the fluid circulating in the tissue inter-spaces (122). Cesaris Demel's views represent an amplification of Wright's theory and though difficult of experimental demonstration, have much merit, for they harmonize many of the conflicting points regarding the nature of platelets. Strong indications that such is the mechanism of thrombopoiesis may be seen in the spleen of asphyxiated kittens, although even Cesaris Demel admits that they are not entirely satisfying. Endothelial elements of the spleen possess the property of condensing protoplasmic masses about themselves and even endothelial elements of vessels such as the aorta, may, under certain conditions, develop it (424).

The pseudopods of megakaryocytes were not considered by Wright (681) to be passive structures but evidence of vital activity, for he observed ameoboid movement not only in megakaryocytes but in blood platelets. Wright saw the hyalin marginal zone of megakaryocytes and platelets constantly changing its outline, sending out and *withdrawing* short processes of various shapes. There is additional evidence, however, that for the actual formation of pseudopods and platelets, the action of the blood current is necessary. In tissue cultures, megakaryocytes present very little motility, no phagocytic activity and no appearances that could be interpreted as the formation of platelets (637). Since cellular motility and phagocytic activity are usually exalted in tissue cultures, it would appear that megakaryo-

cytes do not possess either. The megakaryocytes observed, "in vitro," fragmenting into platelets, appeared to Sabin (556) and Bedson and Johnston (55) to be undergoing a disintegration process and not an active formation of pseudopods with fragmentation. Failure to demonstrate motility and platelet formation in tissue cultures of megakaryocytes does not mean necessarily that it is lacking "in vivo," for hormonal or humoral factors may be needed for thrombopoiesis.

Rate of production. Megakaryocytes seem to have a capacity for rapid production of platelets. A few hours after an intravenous injection of peptone into animals, Seeliger (582) observed signs of increased thrombopoietic activity in the bone marrow. Injections of pyrocin and lead into cats, rats, and guinea pigs bring about an increase, in a few hours, of the number of bone marrow megakaryocytes with a cytoplasm made up almost entirely of platelet-like material; normally such megakaryocytes are rare in adult bone marrow (212). During the thrombocytosis which follows asphyxia induced in kittens (73), the spleen of the animals shows an increase in the number of megakaryocytes and a marked accentuation of their thrombopoietic activity (122), abnormally large forms breaking off from the cytoplasm. The thrombocytosis of asphyxia is due both to an increase in production of platelets and to the splenic contraction that takes place during suffocation of the animal (122). In dogs that received intraperitoneal injections of antiplatelet serum, the size and percentage of macro platelets in the peripheral blood begins to increase 3-4 hours after the injection while the total number of platelets is decreasing but before they have entirely disappeared (626); the abnormally large forms which precede any significant rise in the number of platelets gradually disappear as the platelet level becomes stabilized (Chart 1). These facts seem to indicate that there is a prompt response in the thrombopoietic tissues to a diminution in the number of platelets in the blood. The mechanism responsible for the acceleration or reduction in the activity of these tissues is not known. A moderate lipemia, manifested principally by significant rises in the plasma fatty acids and lipid phosphorus accompanies the acute and reactive stages of the thrombopenia induced in dogs by injections of antiplatelet serum (628a), a circumstance that may indicate that the plasma lipids are somehow intimately connected with the mechanism of platelet regeneration and destruction.

The rate of production of platelets is such that the entire number in the circulation may be replaced between 3 and 5 days. Platelets are produced at the rate of about 100,000 per c. mm. of blood per day, after the first signs of beginning regeneration appear. This rate of regeneration may be observed after the thrombopenias of acute infection or those induced in rabbits, guinea pigs, dogs and man by blood defibrination (176) (218), antiplatelet serum (53) (626) (624) or saponin (218). Under abnormal conditions as, for instance, immedi-

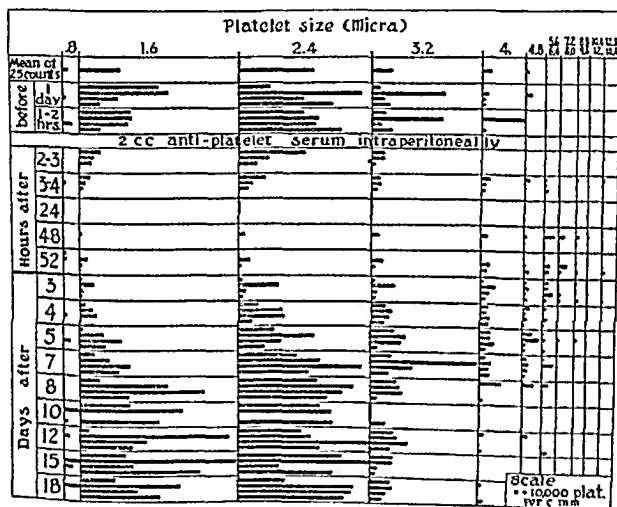


CHART 1. CHANGES IN SIZE (LENGTH) OF THE BLOOD PLATELETS OF FOUR DOGS AFTER THE ADMINISTRATION OF ANTIPLATELET SERUM (ONE COUNT LOST ON 10TH DAY)

ately after splenectomy in patients with a long-standing thrombopenia, the rate of output may be greatly accelerated. Increases of 400,000 to 1,000,000 per c. mm. within 24 hours have been observed (634).

Abnormal thrombopoiesis. The process of thrombopoiesis may be accelerated or altered by a variety of causes. The intensity of response of thrombopoietic tissues differs and is not always conditioned by the degree of the stimulus (243). When accelerated, the

process of differentiation and fragmentation is altered, resulting in the production of portions unequal in form, volume and staining qualities. Long platelet forms may be found in the peripheral blood resembling terminal portions of the pseudopods of megakaryocytes, great round masses with variable amounts of hyalomere and chromomere, and large masses that may retain their property of fragmentation after being freed in the circulation (Plate 1, Fig. 11, 12). These abnormal forms are often found in the leukemias and chronic thrombopenias and in conditions in which there is great alteration in the myeloid tissues and an increase in the number of megakaryocytes. In thrombopenias associated with pernicious anemia and aplastic anemia and especially in the latter, the number of bone marrow megakaryocytes is decreased (243). In the thrombopenia of lymphatic leukemia and aleukia hemorrhagica, they are also *decreased* and sometimes entirely absent (243) (582). An increase in the number of these giant cells in medullary and extra medullary tissues is observed in polycythemia vera, hemorrhagic anemias, (243) and in certain tumors (314).

In chronic thrombopenia ("essential thrombopenia"), the bone marrow may often contain a normal (582) (243) (385) or even increased number of megakaryocytes (582) (232) (160). Little attention, however, has been paid to the duration of the thrombopenia, to the type of these megakaryocytes and the stage of their differentiation when attempting to correlate the number of the latter with numerical changes in platelets. In pernicious anemia, the bone marrow is likewise hyperplastic but most of the erythroid elements are of the primitive type, in the early stages of differentiation. In rabbits kept thrombopenic by Firket (218) from 6 to 8 days, by injections of saponin, nearly all megakaryocytes were in the lymphoid stage. If the thrombopenia were produced by repeated withdrawals of blood, defibrination and reinjection, the blood platelets would recover their original level in 3 days and in these animals the marrow megakaryocytes were of the mature type, with the cytoplasm filled with azurophilic granules (218). When active regeneration of platelets is taking place, maturation is accelerated (271), the differentiation of megakaryocytes is extensive and complete, and half of the cells are almost entirely stripped of cytoplasm (107). When regeneration is not active

(as in prolonged experimental thrombopenia) the differentiation is arrested at the lymphoid stage (218). According to Kaznelson (347), in chronic thrombopenic purpura there is no disturbance in the regeneration of platelets, and, indeed, this regeneration is apparently even more active than normal; the thrombopenia in the peripheral blood is due to increased destruction of platelets by the spleen. The spleens of many individuals with chronic thrombopenia contain a higher number of platelets than normal spleens (582). Strong indication that the primary disturbance in this condition is in this organ is the fact that following splenectomy there is often permanent recovery from the symptoms of bleeding. The spleen is closely involved in the storage, distribution, utilization, and sometimes even in the production of the platelets (121). This organ may exert an inhibitory effect on megakaryopoiesis, for its removal in some animals is followed by proliferation of megakaryocytes (520). Torrioli and Puddu (637) demonstrated that megakaryocytes in tissue cultures maintain a normal aspect until they are placed in contact with blood or extracts of organs rich in reticulo-endothelial tissue, especially the spleen. These organs seem to elaborate a toxic substance which is continuously being thrown into the circulation and destroyed or neutralized as it goes through the body (637). The elaboration of this substance is pathologically exaggerated in individuals with chronic thrombopenia ("essential thrombopenia"). Torrioli and Puddu (637) suggest that the increase in the number of platelets that follows splenectomy in thrombopenic purpura is due to the removal of an organ containing a large amount of tissue producing a megakaryotoxic substance and that the relapses that sometimes occur may be traced to production of this substance in other organs of the body. In certain instances removal of the uterus has been followed by disappearance of the symptoms and the thrombopenia (447). Direct evidence of the presence of a thrombolytic substance in the spleen of these patients has been advanced in the as yet unconfirmed report of Troland and Lee (640a).

The production of platelets may, under abnormal conditions, take place in the peripheral blood, where it seems to occur normally in mammals (191). Megakaryocytes appear in the peripheral blood (thrombocythemia) almost invariably in the acute phase of leukemias (580) (466) (120) (156) (346) (445) (556) (205) and occasionally in

the "aleukemic" phases (466). The cells may consist of only a single naked nucleus, large or small, or of multiple nuclei, with the cytoplasm breaking up into platelets (158) (13). On Plate 1, Fig. 7, are illustrated 2 megakaryocytes found in the blood of a patient with a pernicious leukothrombopenia. The giant cells have also been found in the peripheral blood of patients with pernicious anemia, "essential thrombopenia" (347) lead anemia, polycythemia vera (466), simple leukocytosis (158), lobar pneumonia, Hodgkin's disease, and sepsis (445). Percentages from 1 to 8 percent in leukocyte counts of from 50, to 150,000 have been observed (445). In nearly all instances, the number of platelets was increased and there were abnormally large forms. In moderate cases of myeloid leukemia the appearance of megakaryocytes in the blood and an increase in the number of platelets *may be the first sign of increased activity of the disease*; a constant relationship between the number of megakaryocytes and platelets may be maintained in the blood (445). X-ray treatments reduce markedly the number of the giant cells (231). Splenic and hepatic megakaryocytes enter the peripheral circulation more easily than those from the bone marrow (203). In the lungs of patients with myeloid leukemia, or Hodgkin's disease, great numbers of megakaryocytes may be found (445) (107), much larger than those found in the peripheral blood before death (445). There is strong indication that megakaryocytes appear in the lung capillaries in large numbers in conditions calling for abnormal hematopoietic activity (23) (681) (107). That their source is wholly embolic may be questioned, in view of new interpretation given to their presence in the lung by Howell and Donahue (310). Repeated auricular punctures in leukemic patients have shown, however, a greater number of megakaryocytes in blood from the right auricle than from the peripheral circulation (13), an indication that at least some of the giant cells are actually transported to and arrested in the lung capillaries.

THE DISTRIBUTION OF PLATELETS

In the tissues. Except for the lumen of vessels, the spleen is the only organ in which platelets may be demonstrated in the guinea pig, rat, mouse, pig, cat, rabbit, dog and man (225) (407) (508) (121). It is difficult to demonstrate platelets in the bone marrow (225) (407),

perhaps on account of the nearness of megakaryocytes to the blood vessels where the platelets are broken off and carried away. In the spleen of the rabbit, platelets are found in groups of 5 to 15, outside of the follicles (407). In the dog they are more numerous, and in man they are normally present, always outside the follicles, usually in the sinuses or between the cells of the pulp. Injection of platelet suspensions intravenously into rabbits does not seem to alter the number of platelets in the spleen; bleeding gives rise to a marked increase and injections of anti-platelet serum, to a temporary diminution (407). Platelets are abundant in the spleen in pneumonia, and rare in typhoid fever (407).

In the vessels. The distribution of platelets in the vessels of different organs seems to vary widely. There are variations not only between vessels of different portions of the body but in the same vessel within short periods of time. Busatti (109) pointed out that a thrombocytosis in the peripheral blood may not be accompanied by an equivalent increase in the number of platelets in the vessels of internal organs. In the rabbit ear it is possible to watch a group of small vessels with a moderately active circulation for a minute, sometimes longer, and see only an occasional platelet passing through; again, "schools" of them may come through so that the plasma contains many platelets for an equally long time (56). Such inequalities in distribution must increase the error of platelet counts done on small amounts of blood; if the count is estimated indirectly (from the ratio of erythrocytes to platelets) the possibility of error must be even greater. According to Poletini (508), platelets are rarely observed in the vessels of tissue sections of the brain, heart, kidneys, adrenals, and thymus of normal guinea pigs, cats, rabbits, pigs, rats and mice killed instantaneously by puncture of the medulla and examined immediately afterwards. In normal adult guinea pigs, rats, pigs, and mice, platelets are frequently found in the capillaries and great vessels of the lung and liver, and in great numbers in the liver vessels of fetal or pregnant guinea pigs. In the lung, the entire contents of a vessel may be made up of platelets (508). In the organs of rabbits, platelets are not as easily demonstrated as in cats, but they are also found in the lung and liver vessels. In kittens, platelets are numerous in the small and large vessels of the lung, liver, spleen and pia mater (508). Continuous

perfusion of the lungs and liver of cats brings out relatively more platelets than erythrocytes as the perfusion progresses; the increase is most marked in the lung (310).

Determination of the number of platelets in the efferent and afferent vessels of the spleen and other organs has yielded discordant results. This is not surprising when one considers, among other things, the abundance of platelets in the spleen itself, the irregularity in the contractions of the organ and the variable effects of surgical manipulations involved in the determinations. Moreover, the number of platelets in any vessel is a resultant of many factors: the general rate of platelet regeneration and utilization, inequalities of distribution due to vasomotor changes, concentration or dilution of the blood, and sequestration or mobilization of formed elements in stagnant areas. Electrical stimulus of the vagus in dogs produces, about one minute afterwards, a diminution in the number of platelets in peripheral venous blood and an increase in arterial blood, caused, perhaps, by stagnation of platelets in the capillaries; excitation of the sympathetic at the neck gives opposite results (141). Cori (130) and Howell and Donahue (310) found fewer platelets in blood from the splenic vein than in arterial blood, while Holloway and Blackford (299) found the differences insignificant. Myers, Maingot and Gordon (465) found a markedly smaller number of platelets in the splenic vein in two cases of thrombopenic purpura, while Cumings (139) could not demonstrate any significant differences in an equal number of patients. In normal rabbits, König (364) found three times as many platelets in the vein as in the artery while Tsunashima (642) found them far less numerous in the vein. According to Howell and Donahue (310), in cats, blood entering the lungs through the pulmonary artery contains fewer platelets than that in the left auricle, aorta or carotid arteries. Unpublished experiments of Tocantins and Bradshaw in living cats with the heart and great vessels exposed by Drinker's method (173) failed to demonstrate a constantly higher number of platelets in blood leaving the lungs, as compared with that entering them.

In normal men Tocantins (629) found that the number of platelets in arterial blood of the upper extremity was significantly higher than in venous or cutaneous blood. The differences are markedly accentuated.

ated under pathological conditions (310) (629), and are greater in the winter than in the spring of the year (632). No similar significant differences may be found between the erythrocytes in the same vessels (632). The number of leukocytes in venous blood, however, is said to be, normally, significantly higher than in cutaneous blood, the difference being greater when there is a leukocytosis (448).

Numerical variations. Physiological. In man the number of platelets has been determined most often on cutaneous or venous blood from the arm.³ There are significant differences between platelet counts done almost simultaneously on venous and cutaneous blood (370) (629); the differences are greatly accentuated under pathological conditions and vary in degree from the winter to the summer of the year (632). On 40 young, adult, white, male individuals examined in Philadelphia during the winter and spring of the year, the average number of platelets on blood from the arm was: (1) cutaneous blood $250,000 \pm 7,458$ (Probable Error) ($\sigma = 58,500$); (2) venous blood $310,000 \pm 11,937$ ($\sigma = 110,750$); (3) arterial blood $350,000 \pm 13,889$ ($\sigma = 128,000$) (629).

In Tables 2, 3 and 4 are listed figures for the number of platelets in venous and cutaneous blood, found by authors in various portions of the world, employing different methods and solutions. The averages for the largest series of observations on cutaneous blood (111) and on venous blood (370) correspond very closely to those found in our group. For reasons set forth elsewhere (630), it would seem that figures above 500,000 are seldom, if ever, obtained in cutaneous blood under normal conditions and are perhaps the result of a defective technique. The discrepancy between the figures given by various observers may be more apparent than real. The results of Laker's (379) counts on guinea pigs, Sooy and Laurens' (592) on rats, Casey and Helmer's (113) on animals, Windfeld's (675) and Jurgens (338) figures for human plasma are often found grouped together in the literature as representing the normal number of platelets per c. mm. of blood in man. Climate and seasonal variations, race, time of the day, bodily activity, and the menstrual cycle in women are among the many factors that may have significantly influenced the results of the

³ Unless otherwise qualified, all subsequent statements on the blood platelets of man refer to the number per c. mm. of cutaneous blood from the upper extremity.

TABLE 2

Platelet counts on venous blood by the direct and indirect methods

NAME AND YEAR	AVERAGE $\times 10^3$	MINIMUM MAXI- MUM $\times 10^3$	NO. OF COUNTS	NO. OF SUBJECTS	REMARKS
Direct methods					
Thomsen, 1920.....	*	206-413	*	*	Sol.: sod. citr.; normal salt.
Gram, 1920.....	*	300-550	*	27	1st Sol.: citrate. 2nd Sol.: normal saline. Modified Thomsen's method.
Buckman and Hal- lisey, 1921.....	284	246-328		9	Sol.: distilled H ₂ O; sod. citr.; toluene red; crystal violet; formalin.
Kristenson, 1924....	294(M) 291(F)	204-395(M) 214-360(F)	*	138(M) 20(F)	Sol.: urea; sod. citr.; sublimate; bril. cr. bl.; H ₂ O. Spec. syringe.
Reimann, 1924.....	350	*	*	*	Sol.: citr.; normal saline. Modif. Thomson's m.; blood sedimentation in syringe.
Teoumine, 1927....	*	351-449	10	10(F)	Sol.: citrate.
Windfeld, 1930.....	*	351-531†	60	6	Sol.: sod. citr.
Gutstein, 1932.....	245	*	13	13	Sol.: Nile bl.; MgSO ₄ ; H ₂ O.
Tocantins, 1936....	310	150-690	40	40(M)	Sol.: Formalin; citrate.
Indirect methods					
Aynaud, 1910.....	216	183-252		8	1st Sol.: citr. sod. 2nd Sol.: NaCl; sod. citr.; distilled water; formal.
Cramer and Banner- man, 1929.....	*	250-400	*	*	Sol.: sod. citr.; NaCl; formalin; bril. cr. bl.; H ₂ O.
Preiss, 1932.....	350	320-450	*	*	Sol.: heparin; Tyrode.

* Not stated.

† Per c. mm. plasma.

(M) Males.

(F) Females.

TABLE 3

Platelet counts on cutaneous blood by the direct method

NAME AND YEAR	AVERAGE × 10 ³	MINIMUM MAXIMUM × 10 ³	NO. OF COUNTS	NO. OF SUBJECTS	REMARKS
Hayem, 1878.....	255	200-346	6	6	Sol.: iodized serum.
Cadet, 1881.....	257	*	108	31(F) 77(M)	Sol.: Hayem's sol. B.
Afanassiew, 1884.....	*	200-300	*	*	Sol.: peptone; NaCl; methyl violet.
van Emden, 1898.....	245	208-269	8	5	Sol.: osmic ac.; chromic ac.; glacial acetic ac.
Helber, 1904.....	228	192-264	24	24	Sol.: metaphosphate sod. Special count, chamber.
Wright and Kinnicutt, 1911.....	297	226-367	40	9	1st sol.: bril. cr. bl. 2nd sol.: pot. cyan.
Maixner and Decastello, 1915.....	251	*	*	*	Sol.: sod. metaphosphate. Helber's chamber.
Ottenberg and Rosenthal, 1917.....	*	200-400	*	*	Sol.: sod. citrate.
Schenk, 1921.....	230	174-281	12	6	Sol.: NaPO ₃ ; Formalin; NaCl; H ₂ O. Spec. pipette.
van Herwerden, 1921.	*	217-326	*	*	Sol.: urea; H ₂ O; NaCl. White bl. cell pipette.
Spitz, 1921.....	270	240-300	*	50	Sol.: sodium citrate. Adaptation of Thomsen's method.
Goadby, 1930.....	*	199-393	8	4	Sol.: sod. citr.; NaCl; bril. cr. bl.; Formalin.
Barbieri, 1931.....	*	350-500	*	*	Sol.: H ₂ O; sod. citr.; NaCl; MgSO ₄ ; Formalin; methylene bl.
Jurgens, 1934.....	650†	450-800†	256	256	Sol.: sod. citr.; Micro meth. modif. Thomsen's.
Tocantins, 1936.....	250	180-358	40	40(M)	Sol.: Formalin; citrate.

* Not stated.

† Per c. mm. of plasma.

(M) Males.

(F) Females.

various observers, granting that the methods themselves were adequate.

In Table 5 are listed the averages and range of variation of the num-

TABLE 4

Platelet counts on cutaneous blood by the indirect method

NAME AND YEAR	AVERAGE $\times 10^3$	MINIMUM MAXIMUM $\times 10^3$	NO. OF COUNTS	NO. OF SUBJECTS	REMARKS
Fusari, 1886.....	200	180-250	*	*	Sol.: osmic ac.; NaCl; methyl viol.
Brodie and Russell, 1897.....	635	*	5	*	Sol.: glycerine; absolute alcohol; distilled H_2O ; NaCl; ammonium oxalate.
Determann, 1898....	1/22†	1/18-1/30†	*	110	Sol.: NaCl; K bichromate with methyl viol.
Kemp and Calhoun, 1901.....	833(F) 862(M)	*	75	19	Sol.: Formaldehyde; NaCl; tinged with methyl green.
Pratt, 1903.....	*	217-496	8	2	Sol.: sod. metaphosphate.
Pratt, 1905.....	469	226-725	25	25	Sol.: sod. metaphos.; NaCl.
Sahli, 1905.....	*	200-300	*	*	Sol.: $MgSO_4$; osmic ac.; methyl viol. or methylene blue.
Port and Akiyama, 1912.....	239	201-304	9	1	Sol.: NaCl; gelatin; sublimate; eosin.
Fonio, 1912.....	234	130-350	30	30	Sol.: $MgSO_4$; Giemsa; methyl alcohol. Dry smear.
Degkwitz, 1920.....	300	263-360	*	21	1st Sol.: H_2O ; $NaPO_4$; NaCl; Formalin. 2nd Sol.: H_2O ; NaCl, Formalin; Na_3PO_4 .
Pagniez and Mouzon, 1921.....	*	238-388	4	4	Sol.: Marciano's fluid.
Flossner, 1922.....	760(M) 682(F)	636-906	75	25	Sol.: Tyrode; sublimate.
Bannerman, 1923....	263	222-354	9	1	Sol.: sod. metaphos.; NaCl; Formalin; distilled H_2O ; methylene blue—light tint.
Leake and Guy, 1925.....	*	280-760	*	*	Sol.: Formaldehyde (U. S. P.); sod. oxalate; crystal viol.; H_2O .

* Not stated.

† Platelet/Erythrocyte Ratio.

(M) Males.

(F) Females.

TABLE 4—*Concluded*

NAME AND YEAR	AVERAGE × 10 ⁴	MINIMUM MAXIMUM × 10 ³	NO. OF COUNTS	NO OF SUBJECTS	REMARKS
Gaspar, 1926 .	250(M) 225(F)	*	31	31	Sol.: MgSO ₄ ; methyl alcohol; neutral distilled water; Giemsa. Dry smear.
von Boros and Kaltstein, 1928..	*	300-600	*	3	Sol.: neutral isotonic sod. citr.; bril cr. bl.
Olef, 1930 . .	619	407-1,108	*	44	Sol.: sod. metaphos.; NaCl.
Mackay, 1931..	392	250-450	30	6	Sol.: citrate salt.
Steinmaurer, 1932.	690	*	10	10	Sol.: MgSO ₄ ; Tyrode. Combination of Flossner's and Fonio's meth. Dry smear.
Dameshek, 1932..	716	500-900	100	100	Sol.: bril. cr. bl; citrate; sucrose.
Cumings, 1933	*	590-760	11	11	Sol.: Tyrode; without glucose and with mercuric chloride.
Horwitz, 1933	*	600-900	*	*	Sol.: Tyrode; without glucose; 1-1,000 sublimate.
Olef, 1935 .	514	437-586	38	38	Sol.: sod. metaphos.; NaCl; dextrose.

ber of platelets per c. mm. of blood in mammalia other than man. In most instances the blood was obtained from a vein. In a group of 13 arterial and venous platelet counts done by the author on the femoral vessels of 8 dogs, the average number of platelets in arterial blood was 549,600, in venous blood, 467,800. The average percent volume of platelets in the blood of calves as calculated from figures given by Roderick and Schalk (536), is 0.43%. The erythrocyte-platelet ratio in the rat is about 6 to 1; in the rabbit, 8; in the dog, 13; in the horse, 32 (26); in sheep and goats, 27 (360); in the cat, 19.4 (634). In man this ratio is about 20 in cutaneous and venous blood and about 17 in arterial blood, during the spring and summer of the year. During the winter the ratio is about the same in cutaneous blood, but it is about 11 in arterial and 14 in venous blood (632).

Daily fluctuations. There is a fluctuation of about $\pm 6\%$ in the platelet count of venous blood at different times during the same day,

TABLE 5

Values for the average length and the number of platelets per c. mm. of blood in mammals other than man

ANIMAL	AVERAGE $\times 10^3$	MINIMUM MAXIMUM $\times 10^3$	LENGTH, μ	NO. OF ANI- MAIS	NO. OF DE- TERM.	REFERENCE	REMARKS
Horse.....	352	254-560	3.2†	10	10	Hikmet, 1927	
Horse.....	335	249-461	3-4	*	*	Weiser, 1922	
Cattle.....	684	542-975	3-4	10	*	Arndt, 1925	
Calf.....	490	*		*	8	Mariconda, 1933	
Sheep.....	441	284-659	2.2†	*	*	Ercegovac, 1936	
Macacus Rhesus.....	267	155-424		6	*	Krumbhaar and Musser, 1920	
Dog.....	461 $\sigma = 141$	188-960		53	173	Tocantins, 1936	Direct meth. Venous blood. (Ear.)
Dog.....	467†	353-535	3.0†	17	17	Aynaud, 1909	Arterial blood. (Femoral.)
Dog.....	492	298-793	2-5	10	*	Arndt, 1925	
Pig.....	403	296-616	2.2†	5	5	Hikmet, 1925	
Cat.....	493	368-712	3-6	10	*	Arndt, 1925	
Cat.....	345†	164-500		7	7	Field, 1930	
Cat.....	519	356-760	2-4		*	Weiser, 1922	
Rabbit.....	532 $\sigma = 124$	170-1,120	2-5§	148	991	Casey and Ro- sahn, 1932	Direct meth. Male animals.
Rabbit.....	652 $\sigma = 99$	*		67	127	Backman and coworkers, 1924	
Rabbit.....	536†	424-586		11	11	Aynaud, 1909	Arterial and Venous blood.
Guinea pig...	719	550-888		10	*	Watabiki, 1917	Direct meth.
Guinea pig...	638	584-856		9	12	Ledingham, 1915	Ear blood.
Guinea pig...	783	525-900		4	8	Tocantins (un- publ.)	Ear blood. Di- rect meth.
Rat.....	454	190-760		72	*	Yamamoto, 1933	Stain. Smear method.
Rat.....	477			13	*	Schechet and coworkers, 1935	Direct meth.
Rat.....	795	620-950		8	*	Ma, 1932	
Rat.....	1,208	1,077-1,585		7	7	Bedson and Zilva, 1923	Indirect meth. Tail blood.
Mouse.....	1,800	*		*	*	Reimann and Julianelle, 1926	Indirect meth. Tail blood.

σ = Standard deviation from the mean.

* Not stated in original.

† Calculated from author's figures.

‡ Values obtained from Kohanawa (1928).

§ Values obtained from Richardson (1904-05).

and between different days (370). According to Degkwitz (147) and Kranzfeld (367), platelets are fewer in number in the morning and increase in the afternoon. Zeller (689) found little difference between counts during the day. The differences throughout the day cannot be wholly attributed to the effect of rest, exercise or feeding (370). In 6 dogs examined 73 times for an average period of 16 days, I found, in venous blood, a variation of $\pm 11.8\%$ from day to day.

Age. In the newborn during the first 48 hours, platelets are fewer in number than in older infants. The figures are usually between 150, and 250,000 (281) (645) (155) (589) (349) (411) (431) (653) (323) (435). Throughout the first week of life the count rises slightly (188) and continues to rise slowly until approximately the age of 3 months after which it becomes stabilized (435). The number of erythrocytes behaves in an opposite fashion (341). In 3 seven day old, nursing puppies the average platelet count on blood from the pads of the feet as found by the writer was 269,000 (min. 204,—max. 340). There is greater variation in size and staining in the platelets of the newborn than of adults (589) (411). Large platelets make up 3–8% of the total count, and basophilic stained platelets 4–6% (653). Prematurely born infants have a lower number of platelets than older infants but not significantly lower than full term infants (431) (436). The number of platelets of premature, newborn infants rises to the normal level more slowly than those of full term infants (589), the rise not being significantly influenced by liver and iron therapy, or iron alone (436). A greater utilization of platelets, incident to the trauma experienced during delivery, may be one of the factors contributing to the thrombopenia of the newborn for a similar decrease in the mother's blood takes place in the first 2 days after delivery. In jaundiced newborn infants, the count is lower than in normal infants but it shows a sharper rise in the first three days (188). In normal infants between 1 week and 1 year of age, the platelet count shows little fluctuation; platelet counts on the venous blood of children between the ages of 2 and 11 years of age show no significant difference from the average for normal adults (688).

Men and women over 60 years of age have fewer platelets in venous blood than younger subjects (370). Demmer (153) found an average of 85,000 platelets per c. mm. in 50 men and 20 women of that age;

only a little less than one-half of the group showed signs of a tendency to bleed.

Race. Comparative figures on the normal number of platelets in various races are scarce. Inada and Kubo (318) found an average number of platelets in normal Japanese of 160,000 (min. 150,000—max. 180,000). In normal adult Mongols, Radkewitsch and Gorikina (521) found an average of 133,000, $\sigma = 2,520$, a figure significantly lower than that among white Russians. The lower number of platelets in the blood of Mongols may act as a predisposing factor to the development of hemorrhagic complications; in nearly all post mortem examinations of tuberculous Mongols, Stefko (603) found evidences of an abnormal tendency to hemorrhage.

Sex. According to Kemp and Calhoun (350), Zeller (689), Flossner (222), and Gaspar (243), the number of platelets is lower in women than in men. Langemeijer (384) found the opposite. In women past 60 Demmer (153) found the number lower than in men of an equivalent age group. The differences pointed out by the various authors have not been established from a sufficient number of cases to eliminate the effects of random sampling, seasonal variations, the menstrual cycle in women, etc. No statistically significant differences were found by Kristenson (370) between the platelet counts on venous blood (Table 2) of adult men and women, below or above the age of 60. There are, likewise, no significant differences in this respect between the sexes in rabbits (487).

Posture. Considering that platelets respond very promptly to variations in the degree of internal and external stresses, postural changes might be expected to play an important part in their distribution. There are significant variations in the number of platelets upon a change in posture (300) (606). Within 15 seconds of suddenly getting up, after resting horizontally for $\frac{1}{2}$ hour, the platelet count of normal subjects is decreased (range of decrease: 20–82%); in moderately thrombopenic men the percentage decrease is even greater (300). There is an increase within 15 seconds of suddenly lying down after a period of quiet standing (300). Leukocytes behave very much like platelets in this respect, but the number of erythrocytes is not significantly altered by sudden changes in posture (300). The available studies (300) (606) were carried out on cutaneous blood from the

finger. Platelet counts on arterial, venous and cutaneous blood in various portions of the body during changes in posture would perhaps throw further light on the extent and mechanism of these changes. The effect of postural changes on the number of platelets is probably a result of alterations in the calibre of the vessels, velocity of the blood, platelet utilization and distribution, etc. They may account in part for variations in the bleeding time and petechial reaction of the skin, done at the same time in various portions of the body, and play a part in the localization of hemorrhagic phenomena, as in purpura simplex and orthostatic purpura. In moderately thrombopenic individuals, in elderly people (in whom a moderate thrombopenia is common), in individuals recuperating from acute illnesses (221), or in women about the onset of the menstrual period, prolonged standing may, by raising the venous pressure in the lower extremities, place such increased stresses on the vessels that petechial hemorrhages are formed. A platelet count performed on the upper extremity at the time, may or may not show a thrombopenia; if performed after the individual has changed posture, it may likewise not show any deviation from normal. In patients with purpura simplex, platelet counts should preferably be carried out in the lower extremities, while the individual is standing and after changes of posture.

Temperature. The effect of simple changes in the environmental temperature on the number of platelets is uncertain and deserves careful investigation. A rise in body temperature induced by external heat or a warm bath is said to cause an increase in the number of platelets in rabbits (147) (487), of short duration, cooling being followed by a decrease (487).

Altitude. Ascension to high altitudes produces a rise in the number of platelets of a greater order of magnitude than that for the number of erythrocytes, in cutaneous (351) (383) and venous blood (40).

Season. In man (351) and in the rat (43), the number of platelets is decreased in the summer. In the winter, in Philadelphia, the number of platelets in arterial and venous blood of man is significantly higher than in the spring; there are no significant differences between counts on cutaneous blood in the two seasons (632). This seasonal diminution may account in part for the greater incidence of thrombopenic purpura in the spring of the year (330).

Physical exercise. According to Behrens (57), physical exercise consisting of rowing over a course 6 kilometers long or running a distance from 200 to 400 meters, always caused an increase (18 to 180 percent) in the platelet count of trained and untrained individuals. The increases were more marked in the untrained men, the trained ones having usually a higher pre-exercise platelet level than the former (57). Isaacs and Gordon (320) estimated that the number of platelets was increased 2-3 times after a foot race lasting $2\frac{1}{2}$ -3 hours over a 26 mile course. In rabbits, physical exercise produces an increase in the platelet count, the increase being directly proportional to the duration of the exercise (487). On the other hand, Caccuri (110) found that physical exercise consisting of turning the wheel of an ergostat for periods of 40 minutes to 2 hours produced a considerable diminution in the number of platelets in cutaneous and venous blood from the arm, immediately at the end of the period of exercise; there was a slight rise from this level 6 hours after, an increase above the pre-exercise level at 24 hours, and a return to normal 48 hours after. Finally, Kristenson (370) found no significant changes in the platelets of venous blood after physical exercise of moderate intensity lasting from $1\frac{1}{2}$ -9 hours or after short ($1\frac{1}{2}$ -7 hours) or long (48-96 hours) periods of rest in bed. Differences in the type and duration of the physical exercise in each experiment and the time of collection and source of the blood, perhaps account for these discrepancies. Since platelets respond very rapidly to an increase in stress, the effect of systematic, well controlled physical exertion (of the entire body or of an extremity only) on the distribution of platelets is worthy of being reinvestigated. There seem to be no studies available on changes in the number of platelets during sleep.

Feeding. Reports on the effect of feeding on the number of platelets are discordant. According to some investigators, no significant changes occur after feeding (507) (155) (514) (289) (352) (513); others claim that feeding is followed by an increase in the number of platelets in man (281) (111) (537) (61) (340), rabbits (279) and rats (43). According to Benhamou and Nouchy (61), an increase after feeding is a constant phenomenon in blood obtained from cutaneous punctures in man; it appears approximately 20 minutes after the ingestion of food, reaches its greatest height in 40-60 minutes, with a return to

normal at approximately 2 hours after and a little below normal, 3 hours after. The increase amounts sometimes to almost twice the number before the ingestion of food but generally is only approximately $\frac{1}{3}$ more. It has a constant, regular rhythm which is markedly in contrast with the irregularity of the "digestive leukocytosis"; it is more marked after ingestion of milk, meat and sugar, less marked after ingestion of fats and insignificant after ingestion of vegetables (61). The changes follow about the same course in children, adults or old people; in the infant, the increase appears regularly after nursing, and at approximately the same time. In diseases of the stomach without obstruction, and in splenectomized individuals there is no change in the curve; in pyloric stenosis and diseases of the liver with hepatic insufficiency, and in far advanced diseases of the spleen, the increase does not take place; in thrombopenias there may or may not be a change in the curve after feeding; in pernicious anemia, during a phase of remission, there is a normal increase. There seems to be no relationship between the increase and the hydrochloric acid content of the gastric juice, no significant change following ingestion of 4 percent hydrochloric acid solution (61). In venous blood the variations after feeding are small and unimportant (370). The increase in the number of platelets that is said to occur after ingestion of food should be controlled with counts of the chylomicrons for the latter are confused with platelets by many workers. A rise in the number of chylomicrons in the blood usually takes place one hour after the ingestion of food and the level may remain high for approximately 4 hours afterwards (590).

A decrease in the number of platelets follows a period of fasting in man (147), rabbits (25) (279), guinea pigs (111), and dogs (25). According to Kristenson (370), no important changes in the venous blood of man follow fasting periods of 14 to 48 hours.

Menstruation. There is general agreement that the number of platelets shows a significant decrease on the first day of the normal menstrual period (501) (486) (60) (340). This decrease may amount to 50-75% of the number present before menstruation, may last into the second day, and does not seem to be a result of the bleeding for it precedes its actual onset by a few hours (340) (501). Towards the end of the period (3d and 4th days) an increase takes place (295) (116)

(486) (60) (340), amounting to about 21% of the normal level, less in order of magnitude, therefore, than the decrease (486). The platelets may continue to increase in number 1-2 days after the cessation of the bleeding (116) (340). Between menstrual periods the fluctuations are unimportant (340). The post-menstrual increase is not as great as in patients that have been bleeding from other causes (295). No significant changes seem to take place at the menopause, in normal women (60).

Pregnancy.—There is no general agreement as to changes in the number of platelets during gestation. Rebaudi (525) found a great increase in the first four months and in the last month just before delivery, while Neu and Doenecke (467) and Benhamou and Nouchy (60) observed only slight increases in the last months of pregnancy. According to other authors, (80) (200) (576) (233), there are no significant differences between platelet counts in pregnant and non-pregnant women before the time of delivery. Kristenson (370) found no changes in venous blood in the later months of pregnancy. Platelet counts at different months of pregnancy should be corrected, among other things, for seasonal variations. A definite decrease in the number of platelets seems to occur in the first stage of labor with a return to the base level during the second stage (200). Blood from the veins of the umbilical cord of the fetus shows about the same platelet content as that from the mother during the second stage of delivery (200). During the first and occasionally the second day after delivery, there is in the mother's blood, a slight decrease (60) (378) followed by an increase (233) (378), which reaches its maximum about 8 days after the date of delivery (60) (200) (312). Similar changes take place after parturition in rabbits (588) and ewes (275). The increase is particularly marked in the presence of complications, like puerperal phlebitis (378). In moderately eclamptic patients, the number of platelets after delivery does not differ significantly from that of normal patients, but in severe cases a marked decrease may be observed (601).

Numerical variations. Pathological. Differences between the platelet content of blood from various vessels make any statement of numerical changes in determinations from a single source of limited significance only. For practical, clinical purposes, a normal platelet

count on cutaneous blood excludes the presence of a thrombopenia. A thrombopenia in cutaneous blood, however, may not necessarily indicate that the number of platelets in arterial or venous blood is also diminished (629). A thrombocytosis on cutaneous blood from a given area may likewise be considerably less in magnitude than on venous or arterial blood from the same area (634). Volumetric measurement of the platelet content of blood is indicated whenever their number is decreased, for the presence of giant platelet forms may partly make up for a deficiency in numbers. Under these conditions, platelet counts alone give only limited information as to the amount of available circulating platelet material.

Disorders of hematopoiesis. Changes in the number of platelets almost always accompany disorders of the hematopoietic organs. In pernicious anemia, there is nearly always a thrombopenia in venous blood (260) (373), though not necessarily in arterial blood (629). During a spontaneously or therapeutically induced remission, there is usually an increase in the number of platelets beginning either before, simultaneously with, or after the reticulocytosis but always before the rise in the erythrocyte count (260) (373) (471), the mode of inducing the remission (iron, blood transfusion, liver) not being associated with any specific response (471). During a relapse, platelets decrease in numbers and become irregular in size. Giant forms are more numerous in the beginning of a remission, to be followed by moderate size forms, in irregularly occurring cycles (471). In severe anemias of the hypoplastic type, there is usually a marked decrease or even a complete absence of platelets in both arterial and venous blood. Giant forms are rarely seen, a point which is of help in the differentiation of this type of anemia from the macrocytic anemias that yield to liver therapy or the anemia complicating chronic thrombopenic purpura. In both the latter conditions platelet counts are often higher on arterial and venous than on cutaneous blood (629), giant platelets may be found, and injections of epinephrin produce an increase in the count (298). Anemias of the hypochromic type, such as those following acute or chronic blood loss, are usually accompanied by a thrombocytosis (531), the "hematoblastic crisis" of Hayem (286). In chlorosis the platelet count is almost always high. There are no characteristic changes in congenital hemolytic jaundice (298); in

obstructive jaundice a moderate thrombocytosis is the rule. A thrombopenia is observed in only about one-half of the cases of Banti's disease (553). According to Rosenthal (538) and Howel Evans (312), the thrombopenic patients recover from the disease promptly after a splenectomy, while those with a normal or increased platelet count are poor subjects for the operation; this distinction has not been supported by the experience of Bryce (100) and Rousselot (553). In the erythroblastic anemias there is generally a thrombopenia. The platelet count is usually unchanged but occasionally increased in true polycythemia, whether primary (erythremia) or secondary (400); four patients under our observation did not show any significant alterations. In the polycythemia that accompanies an erythro-leukemic myelosis, there is often hyperthrombocytosis and megakaryocytosis; two cases reported by Ludeke (418) showed 26 and 19 times, respectively, as many megakaryocytes in the bone marrow as normally found. In myeloblastic, lymphoblastic and monocytic leukemias and in aleukia hemorrhagica a marked decrease is the rule, while in chronic lymphatic leukemia and in multiple myeloma the thrombopenia is usually of moderate degree. In chronic myeloid leukemia and in lymphogranulomatosis (Hodgkin's disease) an increase in the count and size of platelets is often observed (107) (126). There are no notable changes in malignant neutropenia and acute infectious lymphocytosis. Diseases other than thrombopenic purpura, characterized by a tendency to hemorrhage, like hemophilia and the hemorrhagic disorder of obstructive jaundice, may be accompanied by a moderate thrombocytosis (333). In anaphylactoid purpura, whether of the abdominal or arthritic type, the number is either not altered or increased (144), though the platelets are said to be less agglutinable (337).

A type of thrombopenia exists that does not seem to be secondary to any of the known external predisposing causes. It was designated as "Essential" by Frank (232) to distinguish it from the other types. The thrombopenia may last for a number of years and is found among many adults whose history leads one to believe that the disease began in early childhood. "Essential" thrombopenia is seen most often in children after the age of 3 years and especially between the ages of 5 and 9 (334). It has been observed in an infant four months old

(264) and, congenitally, in children born from similarly affected mothers (655) (560). The thrombopenia may become more marked during periods of stress as menstruation, adolescence, menopause, exposure to infection or after the ingestion of certain drugs. The etiology of this type of thrombopenia remains to be adequately explained. Since it nearly always disappears after splenectomy, or ligation of the splenic artery (646), it has been attributed to an increased destruction of platelets by the spleen (345). According to Frank (232) and others (582), however, the decreased number of platelets reflects a defect or delay in their formation by the megakaryocytes, which, though not necessarily fewer in number, are immature and incapable of forming platelets; splenectomy removes an inhibitory influence of the spleen on the thrombopoietic tissues and platelets are again formed in normal numbers. Disordered function of the hematopoietic tissues is so often multiple that it is difficult to state which organ plays the primary rôle in a given condition. Increased destruction of platelets by the spleen might conceivably lead to the appearance and predominance of immature nonfunctioning megakaryocytes in the bone marrow just as chronic loss or destruction of blood may lead to a megaloblastic transformation of the marrow. Establishment of a vicious circle would tend to perpetuate the thrombopenia.

An abnormal permanent increase in the number of platelets, not as a complication of other diseases, has been described. It may be a permanent sequel of splenectomy (98) and has been observed in an instance of atrophy of the spleen (198).

Disorders of endocrine glands. There is, as yet, scant evidence of any influence of the endocrine glands on the distribution of platelets in the peripheral blood. Orchidectomy and ovariectomy in rats is followed by a thrombopenia (43), while after either adrenalectomy or the injection of extracts of the sexual glands, there is a thrombocytosis (43) (585). Some facts seem to suggest the operation of forces of endocrinal origin. A male patient reported by Demmer (152), had attacks of thrombopenia and purpura lasting about 8 days, recurring every month for 6 years. The appearance of thrombopenia and subcutaneous hemorrhages before the onset of menstruation has been repeatedly observed (63). Yearly or monthly recurrences in attacks

of thrombopenia may, however, be simply the result of periodic stresses to which the individual is exposed and of which menstruation is an example.

Tumors. In benign or malignant tumors, changes in the number of platelets follow a pattern analogous to that for chronic diseases in general. During local development of the growth, there are either no changes or an increase (286) (116) (513) (495). An increase is the rule if there is anemia from occult bleeding (473). In carcinoma of the stomach with or without metastasis to the bone marrow, and in advanced stages of malignant tumors of the female genital organs, there is often a thrombopenia (361) (81) (116) (324).

Trauma, Asphyxia. Excessive physical stress or trauma of one type or another nearly always produces changes in the number of platelets in the peripheral blood. In young or adult rabbits, cats and dogs asphyxiation by suffocation or strangulation, and especially drowning, leads to an immediate three or fourfold increase in cardiac, arterial and venous blood, with a return to normal after 24 hours; splenectomized rabbits behave likewise (69). In kittens, the platelet rise following repeated attacks of asphyxia (CO_2 inhalations) gradually diminishes in intensity until finally a decrease instead of a rise takes place (394) (73). The response in rabbits is not constant according to Petri (499) and Osseladore (483). The asphyxia thrombocytosis of kittens is partly mechanical (splenic contraction) and partly due to new formation of platelets. During the asphyxia there is greater emission of platelets into the splenic vessels from megakaryocytes and an increase in the number and volume of these cells in the splenic parenchyma (394) (124). Rabbits living in rarefied air at a low atmospheric pressure develop a thrombocytosis (363). It is possible that the same mechanism governs this thrombocytosis and that observed after asphyxia or ascension to high altitudes. There are, nevertheless, no significant changes in the number of platelets of infants who have undergone moderate asphyxia during parturition (112) or in the course of affections of the respiratory tract (133).

Fractures. Fractures of bones are, as a rule, followed by an increase reaching its maximum between the 4th and 15th day (241) (144). The increase is especially marked after fractures of the neck of the femur (241), the type in which a high incidence of pulmonary embolism

is found (412). In experimental fractures there is likewise an increase in the number of platelets in the blood (449) and of the megakaryocytes in the lung (503).

Operations. Following surgical operations, Caesarian sections in particular, there is generally a decrease in the first two or three days, then a rise beginning about the 6th postoperative day, reaching a maximum about the 10th day, with a return to normal within the following 2 weeks (364) (144) (313) (201). A two or threefold increase over the preoperative level may occur, the degree of the rise appearing to be related to the severity of the operation and not significantly influenced by rest in bed, the anesthesia or bleeding (144). If sepsis complicates the operation the increase is even greater (144). The thrombocytosis is perhaps a result of the absorption of products of tissue destruction (364) (144), for intramuscular injection of freshly ground muscle into rabbits and painting of the skin with iodine or tar is followed by similar changes (364) (425) (605). Although there is abundant evidence that trauma in itself is often followed by a rise in the number of platelets (605), the increase that follows splenectomy in man and rabbits is slightly higher, more lasting and constant and prompter in appearance than after other operations (39) (409). The increase is almost always immediate (220) (654) and rapidly reaches levels seldom approached after any other operation (Chart 2). The results of splenectomy for chronic thrombopenia seem to indicate that removal of the spleen alters in some manner the rate of formation and destruction of platelets (*vide supra*).

Trauma is, however, not always followed by a thrombocytosis. The time of appearance and extent of the postoperative rise in platelets varies in different individuals and is somewhat conditioned by the platelet level before operation. A patient with partial duodenal obstruction, 4 years following a perforated ulcer, had a little over one million platelets per c. mm. in venous blood. In preparation for a posterior gastro-enterostomy, he was given 3 direct whole blood transfusions. The platelet count decreased after the transfusions, became even lower after the operation, but began to increase again 4 weeks afterwards, the time at which it returns to normal after most operations. On the other hand, in individuals whose venous blood platelet count is normally about 100,000, trauma, whether due to an operation

or some other cause, may exaggerate the thrombopenia and throw the subject into a phase of bleeding. The thrombopenia in this instance results perhaps from increased utilization of platelets within a short period of time, coupled with an already inadequate rate of regeneration.

Infections. Hayem (282) was among the first to point out that during the acute stage of infectious diseases there is in general a thrombopenia which gives place at the termination of the infection to

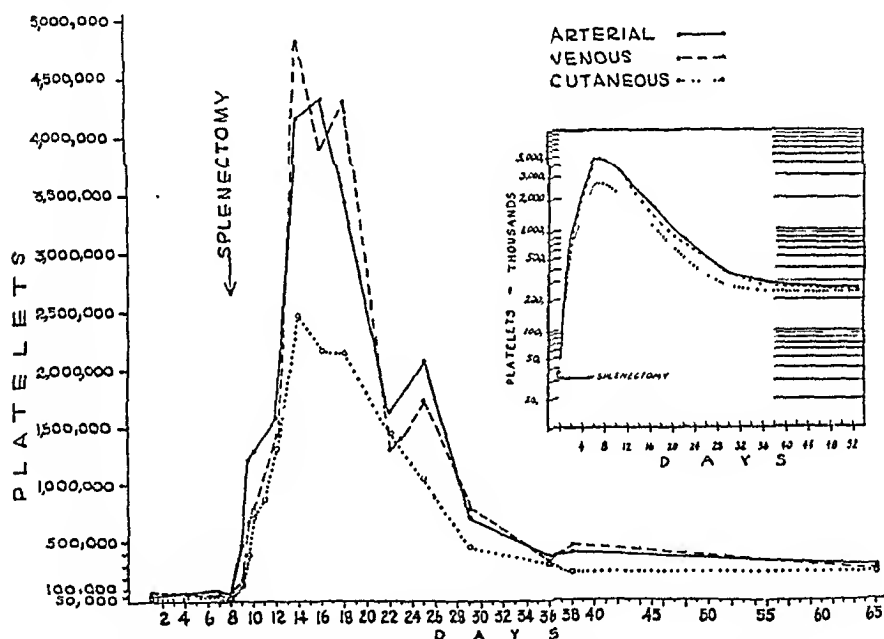


CHART 2. EFFECT OF SPLENECTOMY ON THE NUMBER OF PLATELETS IN THE ARTERIAL, VENOUS AND CUTANEOUS BLOOD OF A PATIENT WITH CHRONIC THROMBOPENIC PURPURA

Inset: The three curves of the main graph plotted on semi-logarithmic ruled paper.

a moderate thrombocytosis. Such is the behavior of the number of platelets in uncomplicated epidemic influenza (261) (356) (318) (147) (591), influenzal pneumonia (261), lobar pneumonia before the crisis (289) (513) (527) (591) (37), typhus fever (289) (10) (529) (599), typhoid fever (682) (513) (524) (688) (36) (318), meningococcus meningitis (431), erysipelas (513) (591), rheumatic fever (11), hemorrhagic small pox (317) (469), vaccinia (293) (168), measles (565) (50) (591), mumps (591), and diphtheria (641) (564) (591). There is no agree-

ment concerning the changes in scarlet fever. According to Schiff and Matyas (566), there are no alterations during the fever stage; other authors (513) (591) (298) (147) observed changes analogous to those in other acute infections, while Stahl (600) and Bonciu (83) found an increase during the acute stage and a decrease during convalescence. The thrombopenia of acute infections may (600) or may not be accompanied by hemorrhagic manifestations (261) (469). In most of the infections the period of thrombopenia corresponds closely to the duration of the fever. Tetanus, however, shows the reverse picture. From the first days of this disease there is a thrombocytosis which increases steadily in the cases ending fatally, the changes in the number of platelets not being related to the fever (591). Other diseases during which there is a decrease in the number of platelets are anthrax (591), dysentery (591), malaria (682) (395) (143) (290) (147) (396) (591), Kala-azar (685) (249) (143), ankylostomiasis (395), relapsing fever (143) (684), uncinariasis, and hemoglobinuric fever (143). The degree of thrombopenia is generally proportional to the severity of the disease. In cases ending fatally there is often complete disappearance of the platelets from the peripheral blood; in those who recover, there is a return to the normal level, which may even be exceeded during convalescence. Blood stream infections (meningococcemia, staphylococcemia) may be accompanied by severe thrombopenia and hemorrhagic manifestations (423) (432).

A thrombopenia may be found in congenital syphilis (688) (431), chronic ulcerative colitis (682), and in the splenohepatic and acute types of tuberculosis (298). In pulmonary tuberculosis the number of platelets varies depending on the stage and type of the disease. In the incipient stage and in healed or inactive chronic tuberculosis, there are no significant changes; in moderately advanced tuberculosis there is an increase, which is generally proportional to the degree of activity of the disease (659) (476) (49) (45) (570) (92) (318), the count being reduced to very low figures when the infection is unusually severe (92) and ante mortem (476). Injections of tuberculin are followed by a decrease in the number of platelets in active tuberculosis while inactive cases show either no change or an increase (147) (570). After a thoracoplasty, artificial pneumothorax or phrenicectomy, there occur increases similar in magnitude to those following surgical

procedures in general (92). No correlation may be demonstrated between the hemorrhagic symptoms of tuberculosis and the number of platelets (37) although among Mongols, who have normally a platelet count below 150,000 per c. mm. (521), pulmonary tuberculosis is nearly always accompanied by hemorrhagic manifestations (603). In extrapulmonary tuberculosis there is little or no significant deviation from the normal number (92) (431) (688). The extent and progress of experimentally induced tuberculosis of the bone marrow of rabbits is accurately reflected in the peripheral blood by, among other things, a decrease in the number of platelets; the onset of recovery is initiated by return of the platelets to normal (162).

A thrombopenia may be present in lupus erythematosus, particularly in the acute, disseminated variety (41) (619) (419). The disease has also been observed to develop in known thrombopenic individuals and to be unaffected by splenectomy (334). Since lymphogranulomatous lesions identical in type to those of the skin have been found in the bone marrow and lymph nodes of patients with this disease (562) (319), the thrombopenia may be due to interference with platelet production.

In acute, diffuse glomerulonephritis, the number of platelets may be slightly or markedly decreased (163), returning to normal as the blood pressure and the proteinuria decrease. In localized, infectious nephritis without edema or hypertension, there is often an increase from the very start (163). Hematuria may be present in both types of kidney involvement and, therefore, does not seem to be a result of the thrombopenia. In true uremia, there is a thrombopenia, most marked when the retention of nitrogenous products is greatest (58) (165).

An increase in the number of platelets usually accompanies suppurative infections. In osteomyelitis due to staphylococci and streptococci and in the empyemas, there are marked increases (682) (688), while in dry or serous pleurisy there are no significant changes (37). A thrombocytosis follows the production of subcutaneous abscesses (267) and the injection of bacillus thrombocytogenes (271) in rabbits. Puerperal sepsis and acute pelvic inflammatory disease, if severe, are accompanied by a thrombopenia, which is persistent in cases ending fatally (620) (116). There is usually a thrombocytosis in puerperal phlebitis, preceding by several days clinical evidence of the disease;

with improvement, the number of platelets is restored to normal (378). In acute and chronic appendicitis and in children with acute bronchitis, pertussis, anterior poliomyelitis or encephalitis, the changes are not significant (267) (431).

There is generally no parallelism between the fluctuations in the number of platelets and leukocytes in these infections. During the period of thrombopenia there may be a leukocytosis, as in pneumonia (527) (37) and typhus fever (529); as the fever abates, the number of platelets begins to increase and the leukocytes to decrease (527) (37). The thrombopenia is perhaps more the result of increased utilization and destruction of platelets than of depression in the thrombopoietic function. The mechanism of the thrombopenia may be analogous to that observed after intravenous injection of micro-organisms into animals. The organisms are clumped by the platelets, and both are rapidly removed from the peripheral circulation to undergo disintegration in the deep organs (254) (512).

Drugs. The response to the administration of drugs varies, of course, depending on the dose and kind of drug administered and individual differences between subjects as to susceptibility to the drug, and the platelet level before administration. Salts or colloidal solutions of the heavy metals (lead, gold, silver, mercury) produce generally a decrease in the number of platelets (25) (328) (212) (95) (331) (665) (519). Injections of neoarsphenamine or sulpharsphenamine produce slight, immediate, but transitory decrease in the platelet count of man and experimental animals (335) (692). The decrease in the number of platelets that follows injections of the arsphenamines (430) (452) (478) (42) has been attributed to the benzene portion of the drug (430) for benzene by itself is capable of producing a thrombopenia in man and animals (464) (179) (583). Epinephrine by subcutaneous injections brings forth an increase within five minutes in normal man and animals (74) (530) or in patients with chronic thrombopenia (21), malaria, typhoid fever, recurrent typhus, various acute infections and alcoholic cirrhosis (59). The response to epinephrine lasts about one hour and does not occur in splenectomized individuals or in those whose spleen shows advanced structural changes (59). It is apparently a result of contraction of the spleen for it reaches its maximum simultaneously with the time when the spleen shows the

greatest reduction in size (437) and the blood pressure is increased (690). Similar changes follow injections of histamine (437) (690) and of a splenic extract (274). After injections of peptone there is a marked but temporary decrease in the platelet count (666) (690) (25) due to massing of platelets into large clumps in the deep viscera. Solutions of 25% colloidal thorium dioxide ("thorotrast") injected into rabbits in doses between 0.5 and 5 cc. per kilogram body weight produce symptoms of hemorrhage and a marked thrombopenia which may later be followed by a thrombocytosis (586) (381). If injection of the drug is repeated while the platelets are below the normal level, the thrombopenia that follows is more marked than when the number of platelets is normal (586). This fact suggests why the injection of even small doses of "thorotrast" or other thrombolytic substances in moderately thrombopenic individuals is followed by a marked thrombopenia and tendency to hemorrhage while in normal individuals such injections cause little disturbance. Clinical use of "Thorotrast" has been sometimes complicated by the development of a tendency to bleed, particularly in patients with cirrhosis of the liver (128) (68) (510). In patients with inflammatory lesions of the abdominal veins, the phase of thrombocytosis may induce a fatal thrombosis (523) (105). A thrombopenia may follow administration of quinine in individuals allergic to this drug (428) (290) (498). The hemorrhagic manifestations and thrombopenia which sometimes appear during the course of malaria may be thus aggravated by quinine.

An increase in the number of platelets follows the administration of pyrocin (509) (426), trypan blue (426), phenylhydrazin (539), or 1% congo red (660). Intravascular injections of india ink, carmine, barium, and quartz particles produce a thrombopenia (615). External applications of croton oil, injections of aleuronat intraperitoneally and turpentine and saponin subcutaneously lead to a reduction in the number of platelets followed by an increase above the normal in rabbits (107). Saponin has a strong lytic action on platelets "in vivo" and "in vitro" (216), and its injection may result in a thrombopenia and appearance of megakaryocytes in the peripheral blood (433).

In the chronically morphinized rat, the number of platelets is greatly increased during the early stage of intoxication. When the

drug is withdrawn, there is a decrease and marked irregularity in size and shape of platelets throughout the entire period of craving. If lecithin is fed to the animals during this period, the number of platelets is markedly increased, while erythrocytes show only slight changes (420).

Ether narcosis, in itself, does not change significantly the number of platelets (25) (144) (227). Rabbits treated with CO and CO₂ inhalations show a two-fold increase in the number of platelets within fifteen minutes, with a return to normal in 12 hours (70) (71). Daily repetition of the inhalations in kittens decreases the response gradually, until finally a diminution instead of a rise takes place and the number of platelets is fixed at a lower level (73). Oxygen inhalations do not cause a significant change in the number of platelets in normal rabbits; in normal, splenectomized, or deplateletized animals in which an increase has been brought about by CO inhalations, oxygen inhalations reduce the number of platelets to normal in one hour. In normal and splenectomized rabbits deplateletized by injections of antiplatelet serum, asphyxia produced by suffocation or by CO or CO₂ inhalations produces an increase in the number of platelets while a subsequent inhalation of oxygen brings about the opposite effect (71). Sestini (584), however, found in the venous blood of normal men and in the heart blood of guinea pigs, that inhalations of pure oxygen for 45 minutes are followed immediately by almost a two-fold increase in the number of platelets, maintained for approximately 3 hours. The disparity between Sestini's and Bianchini's results may be due to the fact that the latter used short inhalation periods whereas the former submitted his subjects to inhalation periods of 45 minutes.

Serums. Intravenous injections of heterologous serum reduce the number of platelets in rabbits, guinea pigs and horses while homologous serum produces a temporary increase (279). In systemic reactions that complicate injections of therapeutic serums there is usually an increase from the 4th to the 12th day after the injection (564) (677). Antispleen, antibone marrow or antiplatelet serums produce a thrombopenia when injected into animals while antilymph gland, antifibrinogen, antileukocyte and antierythrocyte serums are inactive in that respect (52).

Blood transfusions. Transfusion of citrated or unmodified blood

between normal dogs and between rabbits is nearly always followed by a significant decrease in the number of platelets (634) (339); in dogs, as in man, if the blood is incompatible the thrombopenia may be marked, the bleeding time become prolonged (634), and other signs of abnormal bleeding may appear (488) (172). In such cases the transfused, incompatible red blood cells of the donor may act as foreign particles and any available platelets in the donor's as well as the receiver's blood stick to the clumped erythrocytes and leave the circulation in a manner analogous to that after injection of micro-organisms or heterologous erythrocytes. This circumstance may account for the signs of increased bleeding that occasionally follow transfusion in patients with hemorrhagic disease. The effects on the platelets of transfusion for simple blood loss, are more difficult to evaluate, for hemorrhage in itself is usually followed by a thrombocytosis. Destruction of platelets may also account in part for the reactions that sometimes follow transfusions. This is apt to occur especially after slowly given transfusions, the delay causing partial coagulation of the donor's blood and disintegration of platelets (172). The rise in temperature produced in rabbits by injections of defibrinated blood is due mostly to products of platelet disintegration (234). There is no constant or permanent increase in platelets in arterial, venous or cutaneous blood of thrombopenic men and dogs within 24 hours after transfusions of compatible blood (634) (334). The hemostatic effect of blood transfusion in thrombopenic purpura is perhaps due to immediate utilization of the donor's platelets in checking bleeding (634); the remote effects are impossible to appraise with any certainty. By way of contrast, in pernicious anemia, which is seldom accompanied by hemorrhagic disturbances, a remission induced by blood transfusion is followed by an increase in the number of platelets (661) (373).

Vitamins. Deprivation of vitamin A does not seem to lead to important changes in the blood platelets of rats (54) (204) (135). In avitaminosis B there is a higher than normal number of platelets which is reduced to normal levels on administration of the vitamin (355). In scurvy there may be an increase (88) but most often there are no important changes (51) (431). No changes are found in rickets (431) though rats kept in the darkness develop a thrombopenia (136). Irradiated ergosterol, when fed to white rats, produces a thrombocyto-

sis (502); the same drug administered to dogs and normal and thrombopenic men does not lead to significant increases in the platelet count (332).

Irradiation. Exposure of rabbits to ultraviolet rays is generally followed by marked increases in the number of platelets (363) (605) (639). X-rays or radium in small doses, either do not disturb the platelet content of the blood (676) or produce a slight increase (298); in large doses the irradiation brings about a thrombopenia and degenerative changes in the platelets (676) (244) (446) (450) (135). The offspring of rabbits exposed to X-rays (1,400 R units) two or three days before delivery develop a severe fatal thrombopenia and purpura, which is first observed approximately 4 days after delivery (377). Exposure of the spleen of patients with thrombopenic purpura to X-rays in dosages varying from 54 R to 500 R, has no significant effect on the number of blood platelets or the hemorrhagic manifestations of the disease (334 a).

THE UTILIZATION OF PLATELETS

Platelets and physical properties of the blood

Viscosity. Plasma colloid osmotic pressure. Surface tension. The presence of large numbers of platelets alters plasma viscosity only slightly but definitely increases blood viscosity (634), perhaps by furnishing an additional source of internal friction to the erythrocytes. The viscosity of thrombopenic blood is slightly less than that of blood of equivalent protein and cell content (634). In a group of normal and thrombopenic dogs, Tocantins (631) found a low, non-significant correlation between the number of blood platelets and the colloid osmotic pressure of platelet-free plasma. No measurements seem available of the effect of various amounts of platelets on the surface tension of blood or plasma.

Coagulation. Hayem (281) and Bizzozzero (77) were among the first to demonstrate that agglutination and morphological alterations in the platelets progressed almost simultaneously with the development of coagulation in the blood. Platelet alterations are prevented by the same factors that prevent coagulation, such as cold, non-wettable surfaces, antithrombin, alkaline citrates or oxalates, cocaine (174) or heparin. Addition of platelets or platelet extracts accelerates the

coagulation of normal or slowly coagulating blood (286) (86) (134), lymph or hydrocele fluid (86). The greater the amount of extract or platelets added, the more rapid is coagulation and the richer is the coagulum in fibrin (459) (86). Fibrin appears to be deposited in greatest density in the neighborhood of disintegrated platelets, be it in the blood of reptiles (518), crustacea (612), or mammalia (616) (625). Plasma deplateletized by Berkefeld filtration clots very slowly or not at all (134) (181). By using precautions against contamination with tissue juices, Delezene (150) and Hekma (288) collected bird plasma that did not coagulate even when in contact with rough surfaces. The coagulating action of filtrates of staphylococcus cultures is concomitant with their property of agglutinating platelets; absorption with platelet emulsions reduces simultaneously the clot inducing properties of those filtrates (247).

There is abundant evidence, therefore, that platelets influence blood coagulation. It is conceded by most workers that their action consists chiefly in greatly accelerating the process. Opinion is sharply divided, however, concerning: (a) whether platelet disintegration initiates clotting; (b) whether platelets are essential for coagulation. Much of the conflict of opinion on this subject may be traced to the fallacy of speaking interchangeably of the phenomenon of coagulation itself and the inception of coagulation, the equivalent of speaking of combustion and ignition synonymously.

According to some observers (281) (77) (453) (309), coagulation of the blood is initiated by the destruction of platelets. Others (25) (472) (438) (506) have offered evidence to contradict this assertion and point out facts which seem to indicate that platelets are not essential for the development of the process. Agglutination of platelets may take place independently of coagulation (5) and, conversely, many platelets may remain intact in the presence of numerous fibrin needles (7) (390) (625). The latter fact may simply indicate that not all platelets in a given mass of blood are destroyed during coagulation, but it does not necessarily argue against their participation in its inception. Incoagulable peptone blood does not coagulate even in the presence of platelets (6) (505), which again does not constitute strong evidence against the clot initiating action of platelets, for peptone blood is rich in antithrombin and admittedly incoagulable. It is

possible, nevertheless, that a change in the plasma does precede, favor, or precipitate the platelet alterations which initiate clotting. Platelets washed free from plasma are unusually stable; when treated with hirudin for 10-15 minutes, they resist the action of calcium or serum, but completely disintegrate when mixed with plasma (146).

Perhaps both views are correct; under certain conditions destruction of platelets is the initial phenomenon which, so to speak, catalyzes coagulation. Coagulation may, however, be initiated and proceed in the presence of few or no platelets, e.g., coagulation of lymph. The blood plasma, like other sols, may be made to coagulate by the addition of a variety of substances, among which are ether, chloroform, alcohol (305) (442) or simple dilution with distilled water (439). It is possible to demonstrate equally well that platelet alterations precede the appearance of fibrin as well as that some platelets remain unaltered while fibrin is being formed (625). If it is difficult to rule out the possibility that the clot accelerating action of platelets is due to a thin pellicle of plasma adsorbed on their surface (546), it is likewise impossible to eliminate the presence of platelet substances in spontaneously coagulable "platelet-free" plasmas. Precedence of one change over the other depends perhaps on physical conditions surrounding the particular zone where platelets and plasma may be present. If platelets are present in great numbers and happen to fall under conditions that favor their alteration, they will cause changes in the plasma, which in turn will lead neighboring platelets to alter, and so on. A strong indication that such may be the mechanism of the reaction is the fact that both plasma and platelets are very unstable and show alterations under approximately identical conditions. It seems unreasonable to presume that one of them should always, or nearly always, begin to change before the other.

A correlation between simple numerical changes in the platelets and the rate of coagulation of venous blood is often low. Attempts to correlate these two variables "in vivo" seldom yield a high result because the rate of blood coagulation is a function of many variables, including the source and volume of blood observed, the temperature, the type and amount of free and contact-exposed surface, admixture of tissue juices, besides variables in the blood itself, such as the content and quality of the prothrombin, antiprothrombin, antithrombin,

fibrinogen and calcium. In a group of 139 pairs of determinations of the coagulation time and the platelet content of venous blood in dogs with experimental thrombopenic purpura, there was a correlation of only -0.234 ± 0.069 (626). Howell Evans (311) found a "rough parallelism" between the clotting time of cutaneous blood and the number of platelets but Roskam (540) observed an almost constant clotting time of venous blood despite variations in platelets from 35,500 to 400,000 per c. mm. Moreover, a small amount of platelet material (1/20 to 1/50 of the concentration in the original plasma) suffices to restore to normal the coagulability of filtered plasma and accelerate the transformation of prothrombin into thrombin (182). Thus is explained the slight or no delay in coagulation of thrombopenic blood. If the thrombopenia is due to active destruction of platelets, the disintegrated platelet substance may even render coagulation temporarily more rapid than normal (626). In ordinarily slow coagulating bloods like the hemophilic, a thrombopenia may cause even a greater prolongation of the clotting time (624). It remains to be explained, however, why the blood of patients with "aplastic anemia" in whom repeated counts over an extended period of time fail to reveal any platelets, shows only a slight delay in coagulation.

The coagulation hastening action of platelets is apparently due to their property of greatly accelerating the rate of transformation of prothrombin into thrombin. Bordet (86), Mills (441), and Eagle (182) demonstrated that platelets do not contain prothrombin, contrary to the views of Morawitz (453), Bayne-Jones (48), Christie, Davies and Stewart (127), Fuchs (236), and Howell (309). As clot accelerators, platelets behave like cephalin (306) (555), a fact which led Howell to suggest that upon disintegration, platelets liberate that substance (306). The clot accelerating principle of platelets, called "cytozyme" by Bordet and Delange (85), is a lipid, thermostable, soluble in alcohol, ether, chloroform, little soluble in acetone. Extraction of platelets with lipid solvents has, in the hands of some workers (555) (182), yielded only a material with little clot accelerating activity, which makes it probable that the potent fraction is a cephalin-protein complex, far more active than its lipid component alone (306). Other workers (125), however, have isolated from the lipid fraction of platelets a potent clot accelerating substance, pre-

sumably cephalin or cephalin-like in nature containing highly unsaturated fatty acids. The fact that cephalins of vegetable origin are also active clot accelerators is another indication that the active substance in platelets is a cephalin (125). The promising but as yet unconfirmed fact has been brought out recently, that aqueous extracts of defatted platelets possess a clot inhibiting action (125).

Just how the mechanism of coagulation is modified by platelets is still a matter of conjecture. According to Bordet (86), thrombin results from the combination in the presence of calcium ions of the serozyme (prothrombin) of plasma and the cytozyme freed from platelets when in contact with wettable surfaces. Howell (309) believes that the products of platelet disintegration neutralize anti-prothrombin (heparin) thus allowing the transformation of prothrombin into thrombin in the presence of calcium. Fuchs (237) maintains that the lipoid material from platelets neutralizes the antiprothrombin as well as combines with the prothrombin. Mills (438) states that the platelet material unites with calcium and fibrinogen to form fibrin and Fischer (219) that it combines with prothrombin and calcium to form thrombin. According to Pickering (506), platelets cause dissociation of the colloidal complex of prothrombin, fibrinogen, globulin and albumin of the blood. Morawitz (453) believes the platelet substance acts as an organic activator or kinase, and Eagle (184) suggests that it combines with calcium to form a proteolytic enzyme, which reacts with prothrombin to form thrombin.

The view first advanced by Sahli (558) that the delay in the inception of coagulation of hemophilic blood is caused by abnormal stability of the platelets has received the support of some workers (444) (308) (230) (127) (455), while others (9) (359) (207) (506) (183) have been unable to confirm it. According to the latter group, the delay results from the slow transformation of prothrombin into thrombin, either because of a quantitative (207) or a qualitative (9) (183) defect in the prothrombin. The slow availability of thrombin is in turn the cause of the delay in the conversion of fibrinogen into fibrin. Hemophilic prothrombin may be made to change normally into thrombin by the addition of an excess of normal or hemophilic platelets (183). We have, likewise, been unable to find any differences between the clot accelerating and syneresis inducing action of washed platelet

suspensions from hemophilic and normal blood. It is possible, however, that any difference that may exist may be detected only when the original plasma suspension of platelets is used; the handling incident to washing tends, by destroying many of the platelets and freeing platelet material, to equalize the action of both types of platelets. Moreover, functional defects in the platelets are more difficult to demonstrate experimentally than in the prothrombin. It seems probable that the delayed coagulation of hemophilic blood is a result both of a slow disintegration of platelets (with, therefore, slower availability of cephalin-like substances) and a slower conversion of prothrombin into thrombin. There is, normally, a close relationship between the number of available platelets and the rate of transformation of prothrombin into thrombin (182). In hemophilia, it is, perhaps, a summation of defects in these two factors (present in variable degrees in different hemophilics and in the same hemophilic at different times) that is responsible for the delay in coagulation. As Hayem (287) once stated, whatever is responsible for the stability of hemophilic plasma also accounts for the preservation of the platelets in that plasma. An important rôle is conferred indirectly on the blood platelets by the abnormal stability of that plasma in the sense that it opposes or delays platelet disintegration, thereby still further prolonging the inception of coagulation (209).

Structure and physical properties of clots. Platelet-free clots show less adhesiveness, firmness, rigidity, and contractility than platelet-rich clots. Actual measurements of the adhesiveness of clots are not available. The ease with which thrombopenic clots may be removed from containers has been noted by us and others (311). Clots formed over the bleeding skin wounds of thrombopenic dogs either do not adhere to the margins of the wound (623), allowing the blood to flow out around its edges, or adhere so tenuously that even light trauma can displace them (627). Firmness and rigidity are physical attributes difficult of measurement in clots. Comparison of a normal and a thrombopenic clot formed under identical conditions, discloses the fact that the latter does not retain the shape of the vessel when removed from it, and is heavier, softer, more amorphous and jelly-like, more easily displaced and fractured than the normal clot (625). The differences in rigidity, firmness and contractility of

blood clots of different platelet content result partly from variations in structural framework. The fibrin formed in the neighborhood of platelets is in thick bundles twisted about and tied together by large platelet knots (286) (625). Clots rich in platelets yield 20-25% more fibrin per weight than platelet-free clots (459). Kristenson (374) found by quantitative methods, however, that platelet-free clots were firmer than platelet-rich ones, though the latter had a greater elasticity.

The poor retractility of platelet-free clots was first remarked upon by Hayem (285). That the syneresis of blood clots is influenced by the number of platelets in the blood has been abundantly demonstrated (397) (22) (118) (390) (622), though according to some investigators (8) (557) (307) (504) (546) the rôle of platelets is secondary or negligible. Much of this disagreement may be traced to differences in methods of estimating syneresis and the use of this term to denote both the spontaneous retraction of a clot and that following the artificial separation of the gel from the sides of the vessel. Gentle loosening of a platelet-free clot from the vessel wall will induce retraction (307) (504) (546). If such clots are formed under conditions allowing them a large area of free surface, they will likewise show retraction (504) (622). Clots having a large amount of surface exposed to adhesion, however, show little or no retraction unless an adequate number of intact platelets is available (622). Observers inclined to question the importance of the rôle of platelets in syneresis point out that a correlation between the number of platelets in the blood and the degree of clot syneresis is inconstant (674) (465) (421), though Tocantins (626) found a significant, high, direct correlation between the number of platelets and the degree of clot retraction ($+0.759 \pm 0.024$). Differences in technique account for some of these discrepancies. Furthermore, correlations should be established from platelet counts on blood from the same source used for determining clot retraction, that is, venous blood, since significant differences exist between platelet counts on cutaneous and venous blood (629). Moreover, the absolute number of platelets in a given quantity of blood may not give an indication of the degree of syneresis of the clot, for the latter depends on the number of *intact* platelets left after clotting (622). Not all platelets are destroyed during the first and second stages of coagulation (25) (405) (390). Given not unfavorable con-

ditions, blood with an adequate number of platelets may yield an irretractile clot: (a) If most or all of its platelets are destroyed during the first stages of coagulation, (b) if their properties of agglutination and disintegration are defective (250) (337) or have been modified by physical or chemical agents, (c) if the plasma is so altered as to yield a structureless gel (401) (307).

The syneresis inducing property of platelets is not species specific (622). Efforts to extract from platelets a substance which would induce syneresis, have been fruitless (85) (405). In slowly coagulating plasma, Tocantins (625) observed that the platelets converged towards the fibrin needles as these appeared, adhered to them, forming large knots at the intersections. As the knots formed, the fibrin became bent, twisted and shortened. Tocantins concluded that it is perhaps *while* this process is going on and partly as a result of it, that the clot undergoes the visible reduction in volume. The platelet acts then in this respect as a physiologic unit and not through any by-products of its disintegration (622).

Platelets may, therefore, be regarded as playing two distinct rôles in the coagulation of the blood. The two rôles are not incompatible if one considers, not the individual platelet, but the total number of available platelets. When blood is shed, a number of the platelets are destroyed, but a few (those situated in the center of the mass of blood, especially) remain intact. The destroyed platelets accelerate the first phase of coagulation. The intact forms become enmeshed and adhere to the shaft of the fibrin needles as they do when a fine wire or thread is held in the circulating blood stream. Once clumped about the fibrin, the platelets fuse into a solid mass, liberating meanwhile, further clot promoting substances. The clot becomes more and more solid as a result of the binding together of the needles and the increasing deposition of fibrin. Thus, in order to initiate, accelerate and propagate coagulation, platelets must be destroyed; but in order to bind the fibrin into knots, some undestroyed platelets must be available in the neighborhood of the needles as they are formed. This explains why deplateletized blood or blood with a low platelet content will yield a soft, poorly retracted clot, though the rate of coagulation will be only slightly delayed; there is enough platelet substance to promote coagulation but the small margin of platelets

is wiped out during that process and few or no intact forms are left for the binding of the needles.

Platelets and hemostasis⁴

In 1872 Zahn (687) observed that pricking the wall of a vein in the mesentery of a frog was followed by the accumulation and adherence of white corpuscles about the orifice. The mass was in the form of a plug projecting half way into the lumen of the vessel at one end, and bulging into the surrounding tissue at the other. Ten years later Bizzozero (77), after watching the same phenomenon in the mesentery of a mammal, pointed out that this white thrombus was made up almost entirely of platelets. Injury of a vessel wall seems to be followed, in turn, by slowing of the blood current, migration of the platelets from the axis to the periphery, agglutination to each other, and fixation to the injured area (283) (185) (414). If the tail of a tadpole is cut, thrombocytes adhere with great tenacity to the rim of each divided vessel; it is on the strength of the attachment of these cells that the security of the whole plug rests (518). Coagulation of the blood is part of this process, according to some authors (663), while others believe it unimportant or absent (185) (414) (12).

Some of these facts led Hayem in 1882 (283) to predict that a decrease in number or absence of platelets would result in a disturbance of the mechanism of hemostasis. The existence of a thrombopenia in one form of hemorrhagic disease, purpura hemorrhagica, was soon afterwards brought out by Krauss (368) and Denys (154) and confirmed by numerous investigators.

There is abundant direct clinical and experimental evidence to indicate a close association between the number of blood platelets and hemorrhagic manifestations of the purpuric type. Spontaneous bleeding into the skin and mucous membranes often appears when the platelets in cutaneous blood drop below 50,000 per c. mm., and disappear as they return to normal. The transient benefit derived from blood transfusion in thrombopenic purpura results from the utilization by the patient of the transfused platelets of the donor (178) (443) (634). Hemorrhagic manifestations follow the thrombopenia produced artificially in animals by injections of pneumococcus extract

⁴ By hemostasis is meant the spontaneous arrest of bleeding from vessels.

(528), diphtheria toxin, tuberculin and benzol (177), or antiplatelet serum (387) (388) (391) (657). Only serums prepared against platelets or platelet containing tissues (whole blood, spleen) are capable of inducing the extreme thrombopenia and prolonged bleeding; serums against lymph glands, leukocytes, erythrocytes and serum itself are ineffective in this respect (52) (55).

Platelets play an important rôle in endowing blood clots with properties of adhesion and retraction, and the significance of changes in these physical properties of the clot as they affect hemostasis is greater than is generally supposed. The poor adhesive qualities of thrombopenic clots account for the ease with which they are displaced from a wound by the blood pouring out around and under them. An erroneous idea of the speed of clot retraction and of its significance in hemostasis is obtained from observations made with large amounts of blood, in glass tubes. Glass vessels accelerate coagulation but their rigid walls delay clot retraction. In collapsible wall tubes (e.g. cellophane tubes) clot retraction normally follows coagulation almost immediately, the smaller the volume of the clot, the more rapid the retraction. In analogous fashion soon after a clot is formed in an injured blood vessel, *if adherent* to the wall, it draws the ends of the vessel wall together as it retracts. Actual contraction of platelet masses lodged against an injured vessel wall has been observed (12).

Though not denying the fact that platelets play a rôle in hemostasis, some observers interpret the thrombopenia noted in certain hemorrhagic diseases as a coincident or secondary phenomenon. There is no unanimity of opinion regarding the critical number of platelets below which a tendency to bleeding appears. Instances have been reported of normal bleeding time and absence of hemorrhagic manifestations in the presence of a marked thrombopenia (89) (465) (674) (597) (421) and the reverse, normal platelet counts with prolonged bleeding time and evidence of hemorrhagic disease (546) (413) (421). According to Roskam (546), a thrombopenia in itself accounts only for slight prolongations of the bleeding time; marked prolongations result from more complex disturbances as, for example, a delay in the coagulability of the blood. Thrombolytic agents capable of producing purpura also have other deleterious effects; antiplatelet serum has strong leukolytic, hemolytic and local purpurigenic properties (28)

(52) (548). The tendency to hemorrhage that arises in animals that were injected with this antiserum is, therefore, attributed in part to its injurious effects on the vessels (52). Lesions of the vessel walls are considered to be the primary defect in thrombopenic purpura hemorrhagica (52) (546) (421), though actual morphological evidence of the presence of such lesions is far from satisfying.

It seems a hopeless task to define the exact rôle of platelets in the mechanism of hemostasis. The complexity of this mechanism renders it likely that under different conditions the relative importance of its component factors would change. In the disturbance of hemostasis that accompanies chronic thrombopenic purpura, the thrombopenia remains the only thoroughly demonstrated quantitative defect and it seems rational to attribute to it a large share in that disturbance. Failure to find high, significant correlations between platelets and bleeding time is due chiefly to insufficiencies in the methods used by many workers for counting platelets and estimating the bleeding time. Moreover, such correlations should be established from counts performed on arterial blood, with proper allowance for increases in the individual volume of platelets and qualitative changes, and from multiple bleeding time determinations in various portions of the body (627). Much of the reasoning evolved from experiments designed to demonstrate the necessity of vascular lesions in the pathogenesis of purpuric hemorrhages has rested on whether or not *purpura* was observed in the experimental animals. This constitutes an insufficient criterion of the presence or absence of a defect in hemostasis. Bedson's agar serum-antierythrocyte serum experiment (52) seems to prove only what it shows, namely, that if vessels are injured and, simultaneously, the platelets are removed from the circulation, "purpura" follows. It does not prove that in *thrombopenic purpura* a lesion of the vessels must exist along with the thrombopenia. There seems to be no need to postulate the presence of a *pre-existing lesion* of the small blood vessels to account for the hemorrhages of chronic thrombopenia. Nearly every one of these vessels is constantly subject to internal and external stresses that call upon the blood for repair. If the stresses are slight, compensating forces such as vessel contraction, tissue tension or rigidity (628), blood coagulation, etc., may be able to maintain hemostasis, making up for an absence of

platelets. Under greater stresses, however, such as severe local or widespread trauma, infection, or even under moderate stresses but in the absence of compensating forces, hemorrhages will result in proportion to the degree and extent of the stresses. The injection of anti-erythrocyte serum in Bedson's previously mentioned experiment served to add trauma that could not be compensated by the thrombopenic blood. Tocantins and Stewart (633) observed that in the acute stage of experimental thrombopenic purpura, hemorrhages distribute themselves not according to the vascularity of the parts, but in or about organs like the lungs, heart, intestine, and urinary bladder which are undergoing movement almost continually and, therefore, exposed to variable degrees of internal stress.

The properties of platelet extracts or suspensions of intact platelets as efficient accelerators of coagulation of the blood "in vitro" and "in vivo" have led to their employment as hemostatics (229) (369). A critical analysis of the reports on this use of platelet extract seems to indicate they have some value when used locally (252) (549); the results in the control of remotely situated hemorrhages are questionable (252). No rigid criteria whereby the efficacy of these preparations can be judged clinically, have been maintained by most users. Since coagulation of the blood is only a part of the mechanism of hemostasis, the designation of any preparation as an efficient hemostatic must rest on wider grounds than the property of accelerating coagulation alone. A disturbance in hemostasis does not of necessity follow a delay in clotting of the blood. Prolonged bleeding may be a complication of a localized thrombosis; attempts to accelerate blood coagulation under such conditions may only serve to aggravate the bleeding.

Platelets and thrombosis

The original studies of Bizzozzero (77) and Eberth and Schimmelbusch (186) on the formation of thrombi in living vessels clearly showed that platelets are intimately involved in this process. Several factors are, however, necessary for the production of thrombosis of the larger vessels encountered after injuries or postoperatively: stasis of the circulation, changes in the vessel wall and local agglutination of platelets. Circulatory stasis, no matter how produced, favors the

localization of thrombi by allowing the separation and precipitation of platelets from the blood stream. Experimentally, this stasis is better reproduced by partial than by complete occlusion of the vessel. Partial occlusion leads to the formation of eddies and whirlpools and preserves the circulation to the extent that the selective precipitation of platelets from the circulating blood can take place. If a ligature is placed around a vessel so as to rupture the intima, a thrombus forms around the edges of the ruptured portion, thereby constricting the lumen of the vessel. As long as the obstruction is not complete, the blood will continue to flow by; when the increasing mass of agglutinated platelets ("head" or white thrombus) completely obstructs the lumen of the vessel, a column of blood accumulates behind it, upstream to the next bifurcation, forming the "tail" or red thrombus (186). According to Welch (669), changes in the vessel wall are not necessary for the production of thrombi but do help their localization by altering the lumen of the vessel and thereby affecting the circulation.

The precipitating factors in the building up of postoperative thrombi seem to be alterations in the blood and not in the endothelium of the vessel (258) (91). The actual initiating step in the laying down of the thrombus is the agglutination and viscous transformation of the platelets. In the convalescence from certain acute infections and after injuries, operations or parturition, changes occur in the blood that favor the agglutination of platelets. Among these changes are: an increase in proteins with a weak negative electric charge (fibrinogen, globulin), a lowering in the electric charge of platelets (602), and an increase in their numbers. Trauma to the tissues with absorption of the products of tissue destruction is perhaps the stimulus for the thrombocytosis and plasma changes (144) (364), which reach their peak between the 6th and 10th days after an operation, thus coinciding with the average time of onset of postoperative thrombosis (144). Though a thrombocytosis favors the occurrence of spontaneous or artificially induced thrombosis, the actual building up of the white or primary thrombus may be accompanied in the peripheral blood by a sudden decrease in the number of platelets from a high level, the decrease being in direct proportion to the size of the white thrombus (372). Clinical evidence of the thrombosis probably follows formation of the red or secondary thrombus (372) and may appear

only after the number of platelets begins to take a downward course (538) (11).

The agglutination of platelets into large masses may be facilitated by the entrance of certain micro-organisms into the circulation (254). The platelet clumps that form about these micro-organisms may either lodge in the capillaries or, in the presence of circulatory stasis, in a large vein gradually building up a thrombus (258). According to Gengou (247), a certain number of postoperative thrombi not formed in continuity with an inflammatory focus, are due in reality to the action of staphylococci and their by-products. Cultures of staphylococcus aureus freed of bacteria by centrifugation contain two substances, a "staphylocoagulase" capable of accelerating blood coagulation and the agglutination of platelets, and a "staphylotoxin" which brings about their viscous transformation (247). Once in the circulation staphylococci adhere to platelets and through the staphylocoagulase promote a progressively increasing agglutination of these elements which then undergo viscous transformation by the staphylotoxin. These changes are favored by circulatory stasis and an increase in number and agglutinability of platelets. Actual experimental production of such thrombi by the interplay of these factors was accomplished by Govaerts (258). Though bacteria are present in a large proportion of the marasmic type of thrombi (670), sepsis is not always demonstrable externally in association with postoperative thrombosis (144). It does contribute, however, to exaggerate the degree of the postoperative increase in the number of platelets (144).

The pulmonary thrombosis that complicates extensive burns of the skin seems to be partly due to accumulation in the lung vessels of large clumps of platelets detached from the walls of injured vessels (559). Burning the legs of deplateletized dogs is not followed by formation of these thrombi or by symptoms of pulmonary embolism (559).

The thrombophlebitis that occasionally complicates the puerperium, especially after Caesarian section (144) (640), and the venous thrombosis observed in advanced cases of tuberculosis (90) (92) and in the period of convalescence from acute infections are also associated with abnormal increase in the number of platelets. These thrombi seem to differ from the so-called spontaneous types formed in the abdominal

veins, in that the clot is usually palpable, fixed to the vessel wall, and there are signs of venous inflammation and blockage preceded by fever and local pain.

A thrombocytosis, even if marked, does not suffice to bring about thrombosis. A patient with thrombopenic purpura under our observation showed no signs of thrombosis in spite of a venous platelet count of 4,750,000, 6 days after splenectomy. Although, among operative procedures, splenectomy is followed by the highest rises in the number of platelets, there is little evidence to indicate that the incidence of postoperative thrombosis is greater after this operation (493). The exception is Banti's disease, in which splenectomy is not unusually followed by recurrent attacks of thrombosis (312). According to Rosenthal (538), patients with this disease, whose blood before operation shows a thrombopenia, are less subject to thrombosis after splenectomy than patients with a normal or high platelet count. In the former the postoperative rise in platelets is transient while in the latter it may be maintained for several months. Splenomegalic patients with a thrombocytosis probably already have, before the splenectomy, a partial thrombosis or a thrombosing endophlebitis with narrowing of the portal, splenic, or other veins. The operation and the increase in the number of platelets that follows, augments the thrombosis or leads to its extension to neighboring veins.

The accumulation of platelet masses on the mechanically or chemically injured surfaces of veins may be prevented by the administration of hirudin (12) or heparin (65 a). Application of this finding to prevent or reduce the incidence of postoperative thrombosis in man, is now being undertaken with highly purified preparations of heparin (464 a). The decrease in coagulability of the blood induced by the heparin may be only an incidental occurrence; the thrombosis preventing effect of the drug is traceable perhaps to its action in decreasing the agglutinability and viscous transformation of platelets.

Platelets and resistance to infection

A number of facts suggest the possibility that platelets are in some way involved in the reaction of body tissues to bacterial invasion. There is a decrease in the number of platelets during the acute stage of infectious diseases and after the experimental inoculation of animals

with micro-organisms; the increase in humoral immune bodies observed in the convalescence from acute infections is accompanied by a thrombocytosis; platelet thrombi are often observed in the neighborhood of foci of infection; the physicochemical properties of platelets are very much like those of polymorphonuclear leukocytes. It is not inconceivable that platelets, along with many cells of the body, play a part in resisting infection. The difficulty begins when one attempts to analyze and define their exact mode and sphere of activity in this respect.

In 1901 Levaditi (408) noted that some of the cholera vibrio injected into the circulation of a rabbit were phagocytosed by the leukocytes and that some adhered to the platelets. Aynaud (27) observed later that a number of bacteria cause agglutination of platelets, behaving in this respect like peptone and certain colloids. The phenomenon of adhesion of platelets to microorganisms ("platelet loading") has received the attention of several investigators. It may be demonstrated "in vitro" as well as "in vivo," in the presence of serum or plasma. Its modalities differ from species to species and with the type of organism under study. In the rabbit, the intravenous injections of *B. paratyphosus* or staphylococcus is followed by clumping of platelets about the organisms, which disappear from the circulation in 30 minutes; if pneumococci are injected, no adhesion takes place and there is no diminution in the concentration of the organisms in the blood stream 30 minutes afterward. In the dog, injection of *B. paratyphosus*, staphylococci or pneumococci is followed by their agglutination about the platelets. The agglutination of platelets to bacteria is selective: if staphylococci and pneumococci are added together to rabbit blood, only the staphylococci are agglutinated by the platelets (254). Analogous phenomena may be observed after injection of heterologous erythrocytes into mammalia (254). The injection of carmin, India ink or bacterial suspensions into the circulation of birds leads to grouping of the thrombocytes about these substances in a manner identical to that of mammalian platelets (132). "In vitro" platelets stick to and agglutinate about heterologous erythrocytes in the presence of fresh serum or plasma. Plasma alone does not show a very marked action against foreign erythrocytes; platelets must be added in order for the reaction to take place. This

partially explains why heterologous erythrocytes, like certain bacteria and India ink particles, are, when injected into a rabbit, rapidly agglomerated and retained in the capillaries (254).

As to the mechanism of the platelet loading of bacteria and other particles itself, Govaerts (254) attributes the phenomenon to the opsonizing properties of the plasma or serum while Roskam (546) maintains that the opsonization may also be due to the pellicle of plasma surrounding each platelet. Repeated washing of platelets decreases their platelet-loading properties (543). The adhesion, therefore, seems to be a passive process (541) wholly dependent on modifications of the surface of the organism and of the platelet by the surrounding medium. The adhesion takes place between the two coatings of plasma and not between the naked surface of the two particles (546). Platelet loading is essentially similar to the first phase of phagocytosis (393) (254) (541). Like leukocytes, rabbit platelets heated to 56°C. for one-half hour, or kept at 0°C. or treated with distilled water, stick to plasma-prepared bacteria (541).

The adhesion of platelets to bacteria may be the result of precipitation on the platelets and micro-organisms of fibrinogen undergoing flocculation (541). Mixing plasma, platelets and staphylococci is rapidly followed by a flocculation of the fibrinogen and coagulation (358) (262). It is at the beginning of this flocculation that bacteria and platelets precipitate in large clumps. Cold (0°C.) neutral salts of sodium (oxalate, nitrate, citrate, chloride) or chlorhydrate of cocaine (2½%) diminish or suppress the sticking of bacteria or foreign particles to platelets by preventing the opsonization of the particles by the plasma about the platelets (546). These factors also reduce or suspend coagulation of plasma. There are analogies between the phenomena of opsonization and coagulation; both are probably determined (in part at least) by similar or identical plasma changes (546).

Platelet loading of bacteria is not due to the attraction of two particles of opposite electrical charge; *B. coli*, opsonized by plasma, adhere rapidly to platelets suspended in salt solution, both *B. coli* and platelets being negatively charged in this solution (257). As in other instances of bacterial agglutination, the cohesive force overcomes the tendency to repulsion of similarly charged particles (257).

When the blood of a rat that has recovered from trypanosomiasis is

placed in contact with trypanosomes of the same strain responsible for the infection, there is massive agglutination of platelets about the organisms. This phenomenon, first described by Rieckenberg (533), is known as Rieckenberg's reaction and is used for trypanosome strain diagnosis in routine immunological procedure (522). While simple platelet loading of bacteria takes place in plasma or serum from non-immunized animals, Rieckenberg's reaction can only occur in the presence of serum or plasma of immunized animals (270). According to Kritschewski and Tscherikower (375), Rieckenberg's reaction is due to the presence of immune bodies ("thrombocyto-barines") and can only be completed in the presence of complement, while Levaditi's phenomenon may take place in the absence of complement, in normal animals. When dealing with serum from immune animals, addition of complement hinders the phenomenon by causing agglutination of the bacteria among themselves (270). The reaction may be demonstrated with the spindle cells of birds and with the megakaryocytes of mammalian bone marrow (269). It was observed by Kritschewski and Brussin (375a) "intra vitam" in the blood stream and in the peritoneal cavity of mice, followed by or accompanied by lysis or phagocytosis of the organisms. Kritschewski and Tscherikower (375) and Kranz (366) maintain that the fixation of platelets on spirochetes and trypanosomes sensitized with thrombocyto-barines is a physico-chemical phenomenon analogous to the fixation of complement by sensitized antigen. Thrombocyto-barines are said to be present in the plasma associated with the globulin fraction, but their demonstration requires a supply of particulate elements (522).

It is still debatable, however, whether thrombocyto-barines exist in the plasma as distinct antibodies. These substances may simply represent an increased amount of the opsonins found normally in the plasma and responsible for the platelet loading phenomenon with bacilli and cocci (546). The differences between Levaditi's and Rieckenberg's reactions are perhaps only those of degree, accentuated by the size and type of organisms used in each reaction.

It remains an open question whether platelets or their by-products take a part in the destruction of the foreign substances about which they clump. According to Taniguchi, Joogetsu and Kasahara (618), platelets digest those substances by secreting an extra cellular ferment. Frog erythrocytes when injected into rabbits are clumped by rabbit

platelets, the surrounded cells showing evidences of lysis, particularly at points of their cytoplasm about which platelets accumulate. Similar lytic changes may be demonstrated after injections of streptococci, *B. typhosus*, cholera vibrio and, most markedly, after injections of staphylococci (618) (512). Within five minutes after an intravenous injection of staphylococci, nearly all bacteria are found in platelet clumps, whereas phagocytosis by leukocytes attains its maximum only about 30 minutes afterwards. The total number of organisms digested by platelets is far greater than that phagocytosed by leukocytes, which has led Taniguchi and coworkers (618) to conclude that platelets form normally the first line of defense for removal of foreign substances from the blood. The thrombopenia observed after the injection of bacteria into animals is said to be better correlated with the phenomenon of platelet lysis than that of platelet loading (609). The platelets of cats, rabbits and rats, animals naturally refractory to injections of anthrax bacillus, display marked platelet lysis about these organisms, whereas mice, which are little resistant to anthrax, show the phenomenon only slightly (609). Mice inoculated with various organisms (anthrax bacillus, Trypan. gambiense, spiroch. of relapsing fever) after an injection of antiplatelet serum have, when compared with controls (animals inoculated with the microorganisms after injections of normal serum or salt solution), an increased resistance to those infections, due perhaps to the presence in their blood of a great amount of destroyed platelet substance (609). Yet no actual destruction or phagocytosis of foreign particles by platelets, of the nature described by Tait and Gunn (614) and Taniguchi (618), has been observed by Govaerts (254). No proteolytic or lipolytic ferments seem to be present in platelets (47) (546). Any destruction of the platelet lodged particles that may take place, is attributed by Pompesco-Combiesco (511) to the action of plasma substances adsorbed on the platelets and freed by them only upon disintegration. Adsorption of agglutinins on the platelets is what endows them with the physicochemical properties of the agglutinins (593). Platelets from an animal immunized against cholera vibrio, as well as normal platelets placed in contact with an agglutinating serum, undergo a displacement of their isoelectric point towards that of the specific agglutinin (593).

The hypothesis that certain constituents of specific humoral immu-

nity (agglutinins, complement, etc.) originated from platelets (641) (561) (513) has not been supported by analytical study of the question (573) (29) (187) (546). It is well established that serum contains platelet substances in solution (434) and that disintegration of platelets increases the globulin content of the fluid in which they are suspended (129). There is some evidence that bactericidal substances against a limited number of organisms (e.g., anthrax bac.) do exist in the platelets of certain animals such as the horse, rabbit and rat. The platelet-free plasma of these animals, when properly collected, has no bactericidal properties against *B. anthrax* (246), but the serum obtained from platelet-rich plasma possesses them to a high degree and in direct proportion to the number of platelets in the plasma (265). Detailed analysis of these properties by Gruber and Futaki (266), Barreau (47), and Werbitzki (671) established their undoubted presence in the platelets or serum of the horse, rat and rabbit but not of man, dog, ox, sheep, pig, and mouse. Moreover, platelet extracts with anthacidal properties are inactive against *B. typhosus*, *coli*, *dysenteriae* (Shiga), the *staphylococcus aureus*, *streptococcus*, *pneumococcus* and *cholera vibrio* (671). The anthacidal properties of the serum of the horse and rabbit do not, however, confer immunity against anthrax; the course of the disease in these animals is not significantly different from that in animals whose serum lacks those properties (265). Perhaps the trypanocidal properties of human platelet extracts and serum recently demonstrated by Fujibayashi (238) may prove likewise to show no parallelism with resistance to the infection.

There is suggestive evidence that the products of platelet destruction may influence the so-called nonspecific antibody content of the serum (agglutinins against *B. typhosus*, hemolysins against sheep erythrocytes). Injection of antiplatelet serum into rabbits raises the nonspecific antibody content of the blood serum (610) higher than injection of a corresponding amount of normal serum or of various nonspecific substances (686). The fact that "in vivo" destruction of platelets raises the nonspecific antibody content of the serum may account for the occasional increase in the heterophile antibody titer in thrombopenic purpura (65) and for the unusually rapid disappearance of micro-organisms injected into the blood stream of animals

previously treated with antiplatelet serum (104). According to Gyorffy, platelets may, in the presence of complement (by itself inactive) act as nonspecific sensitizers of erythrocytes to hemolysis (273), thus inducing, in this respect, the same conditions for complement fixation as immune amboceptors.

Opinion is divided on the importance of the part played by platelets in ridding the circulation of injected foreign particles. The injection of staphylococci, *B. prodigiosus*, *B. typhosus*, *B. paratyphosus* and a few other organisms into guinea pigs or rabbits is followed by a marked and immediate thrombopenia lasting about 2 hours (27) (254) (618) (175). Delrez and Govaerts (151) pointed out that the injected micro-organisms adhere to platelets and that the mixed clumps of platelets and bacteria are rapidly removed from the circulation. Bacteria which produce fatal septicemia when injected into rabbits, as for example the pneumococcus, do not adhere to platelets or cause any significant change in their numbers (254). These and other facts led Govaerts (254) to attribute to platelets an important antixenic function. Because of their great numbers as compared with leukocytes, platelets can remove organisms rapidly and anchor them in the capillaries of the lung, liver and spleen, thus preventing their dispersion and maintenance in the circulation. In the capillaries, side by side among the platelet clumps, are most of the leukocytes. The destruction of the bacteria is perhaps due to the activity of these leukocytes in the platelet fibrin network enmeshing the organisms (254).

Platelets are, however, not essential for the elimination of bacteria from the circulating blood since a thrombopenia does not retard the disappearance of injected bacteria. Pneumococci, *B. typhosus*, *B. coli*, *B. dysenteriae* and staphylococci are removed even more rapidly from the circulation in deplateletized immune or non-immune rabbits, whether the deplateletization is accomplished with antiplatelet serum (254) (104) or saponin (149). According to Bull and McKee (104), the clumping and disappearance of the bacilli from the circulation of immune animals is directly related to the agglutinin content of the serum; the bacteria seem to agglutinate first and then the platelets stick to them. In non-immune animals the plasma itself may "opsonize" the micro-organisms and render them more

adhesive to clumps of platelets which then collect in the capillaries; in the absence of platelets the bacteria adhere directly to the capillary endothelium (104).

Popesco-Combiesco (512) studied in detail the reaction of cellular defense that follows the penetration of bacteria in the circulation and the rôle played by platelets in this reaction. Although injected typhoid bacilli disappear from the blood in deplateletized animals as rapidly as in normal rabbits, she observed a delay in the process of cellular resorption and digestion in the lung and spleen. In immunized rabbits the bacteria are agglutinated independently from the platelets, which agglutinate separately in large clumps. These two reactions are then followed by the sticking of the platelet and bacterial clumps to each other and their arrest in the lung capillaries. In immunized deplateletized rabbits, the phenomena of agglutination of bacteria, phagocytosis and intracellular digestion do not differ from those in the non-deplateletized rabbits except that no large platelet clumps are seen. Popesco-Combiesco (512) points out that the anti-xenic rôle of platelets consists in their ability to stick to bacteria and, by undergoing lysis, to liberate antibodies (hemolysins, opsonins, agglutinins) condensed from the plasma about their surface, these antibodies then destroying the adherent bacteria. The clumps of platelets constitute a reservoir of antibodies; when arrested at the lung, liver and spleen, they undergo lysis and abandon the antibodies at those places where the inflammatory reaction is most active.

THE DESTRUCTION OF PLATELETS

The duration of a single platelet in the circulation must remain a conjectural subject. It obviously varies under different physiological conditions and depends chiefly on the demands for platelet utilization. Unlike the erythrocyte, utilization of the platelet nearly always means its destruction. Aside from this mode of destruction, a certain proportion of platelets are said to undergo phagocytosis by the endothelial cells of the spleen and other organs, a process which seems accentuated during acute infectious diseases such as typhoid (347) and scarlet fever (64). The morphological evidence in support of this mode of destruction of platelets is far from conclusive, however. Differences in platelet content between blood entering and

leaving the spleen cannot be invoked in its support, for similar differences exist between arterial and venous blood in other locations.

If the rate of regeneration of platelets keeps pace with their rate of destruction, then it would seem that the entire mass of platelets in the circulation is used up every 3 to 5 days. This is approximately the interval of time required for platelets to reach a normal number in the blood of the dog, rabbit, guinea pig, and man, after the first signs of regeneration appear following a spontaneous or experimental thrombopenia (218) (626) (176). The rate of platelet regeneration cannot, however, give an accurate idea of how long a platelet will remain intact in the circulation if it is not utilized. In support of the conception that the life of the platelet is about 4 days, it has been pointed out (444) (176) that the improvement in hemostasis that sometimes follows blood transfusions in thrombopenic purpura (an effect perhaps brought about by the introduced platelets), lasts for approximately that length of time.

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PLATE 1

EXPLANATION OF COLOR PLATE

FIG. 1. Megakaryoblast from the bone marrow of a normal guinea pig.

FIG. 2. Lymphoid megakaryocyte from the embryonic liver of a cat

FIG. 3. Megakaryocyte with cytoplasm undergoing differentiation into azurophilic granules. From the bone marrow of a cat 7 hours after injection of pyrocin.

FIG. 4. Megakaryocyte with cytoplasm entirely differentiated into azurophilic granules. From the bone marrow of a cat, 7 hours after the injection of pyrocin. Figures 1, 2, 3, and 4: tissues fixed and stained with May-Grunwald Giemsa. Reproduced from: Ferrata, A., and Negreiros-Rinaldi, 1915. *Folia Medica*, 1: 815, 839, 863, 891

FIG. 5. Megakaryocyte with pseudopod protruding through the wall of a blood vessel into the lumen. At the extremity of the pseudopod three platelets in the process of development; further down in the blood vessel there are four free platelets. From the bone marrow of a cat; fixed and stained by Wright's method. Reproduced from: Wright, J. H., 1910, *J. of Morph.*, 21: 263.

FIG. 6. Megakaryocyte breaking up into platelets. From the bone marrow of a rabbit. Fixed in 10% Formol in normal salt solution saturated with $HgCl_2$. Giemsa stain. Reproduced from: Downey, H., 1913, *Folia haemat.*, 15: 25.

FIG. 7. Two megakaryocytes, one of which is segmenting into platelets. Observed in the blood of a patient with chronic thrombopenia and leukopenia. Wright's stain.

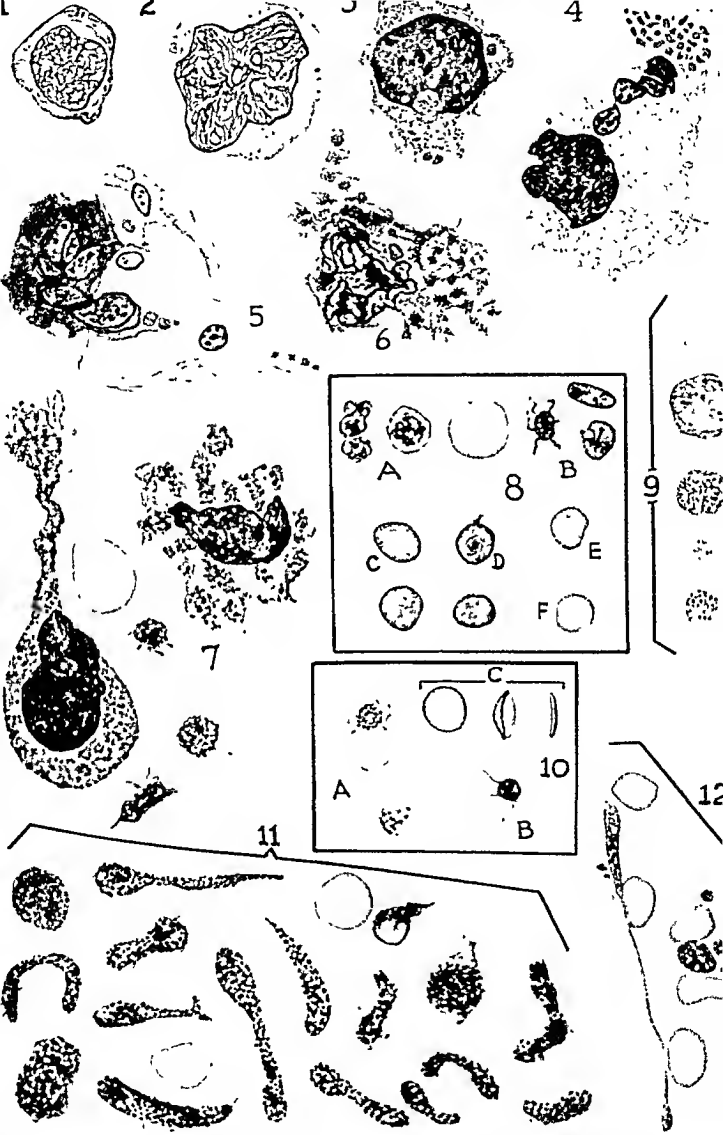
FIG. 8. A. Appearance of normal human platelets in dried, stained blood smears; Wright's stain. B. Human platelets that have undergone more advanced alterations before fixation and staining of dried blood smears; Wright's stain. C. Rapidly formalin-fixed human platelets stained with diluted Giemsa. D. The same as C, stained with 1% methyl violet. E. The same with 1% safranin. F. The same with 1% brilliant cresyl blue.

FIG. 9. Pseudoplatelets observed in the blood smears of patients with leukemia and pernicious anemia. Wright's stain. Drawn to scale in relation to red blood cell in Fig. 8.

FIG. 10. A. Altered human platelets suspended in citrated plasma; "explosive" forms. B. Filamented forms. C. Rapidly fixed normal human platelets, viewed in several positions. From a hanging drop preparation of sub-citrated plasma to which a drop of 0.5% brilliant cresyl blue was added. Drawn to scale in relation to the red blood cell in Fig. 8.

FIG. 11. Macroplatelets observed in dried smears of blood of patients with myeloid leukemia and chronic thrombopenia. Wright's stain.

FIG. 12. Normal and abnormal forms observed in the blood of a patient with pernicious anemia. Wright's stain.



THE RHEUMATIC SUBCUTANEOUS NODULES AND SIMULATING LESIONS

HARRY KEIL, M.D.

*From the Skin and Cancer Unit of the New York Post-Graduate Medical School and
Hospital and the Beth David Hospital, New York City*

OUTLINE

Preliminary remarks.....	262
Historical aspects.....	263
Other lesions formerly confounded with rheumatic subcutaneous nodules.....	265
(a) Froriep's nodules.....	265
(b) Jaccoud's nodules.....	265
(c) Féréol's nodules.....	265
(d) Heberden's nodes.....	266
(e) Miliary nodules of Coates and Thomas.....	268
(f) Osler nodes.....	269
(g) Janeway spots.....	275
Rheumatic subcutaneous nodules in children.....	277
A. Introductory remarks.....	277
B. Clinical data.....	277
C. Evidence for rheumatic nature of subcutaneous nodules in childhood.....	284
(a) Heart involvement.....	284
(b) Joint involvement.....	286
(c) Chorea.....	287
(d) Torticollis.....	287
(e) Salicylate and sulphanilimide therapy.....	288
(f) Association with rheumatic erythemas.....	288
(g) Relation to fever.....	288
(h) Relation to first attack of rheumatic fever.....	289
(i) Age of the rheumatic infection.....	289
(j) Relation to convalescence.....	290
(k) Relation to cardiac failure.....	290
(l) Relation to scarlet fever.....	290
(m) Relation to polyserositis.....	291
D. Prognosis.....	292
E. Some unusual aspects of the subcutaneous nodule in childhood.....	294
1. Dupuytren-like contracture.....	294
2. Calcification and ossification.....	295
3. Dermic nodules.....	297
F. Differential diagnosis in children.....	298
1. Subcutaneous nodules without evidence of rheumatic (heart) disease.....	298

2. Subcutaneous nodules in Still's disease.....	300
3. Gouty tophi in children.....	303
4. Granuloma annulare and erythema elevatum diutinum.....	304
Rheumatic subcutaneous nodules in adults; clinical features, etc. etc.....	306
Differential diagnosis in adults.....	308
A. Subcutaneous nodules in adults without cardiac disease.....	308
B. Subcutaneous nodules in rheumatoid arthritis.....	309
1. Clinical attributes.....	313
2. Pathologic attributes.....	315
A. Pathology of the nodules in rheumatoid arthritis.....	316
B. Pathology of the nodules in rheumatic fever.....	324
C. Comparisons with the Aschoff body in the heart.....	329
D. Experimental studies.....	334
1. Supravital studies.....	334
2. Experimental reproduction of nodules.....	337
C. Juxta-articular nodes of syphilis.....	340
a. Clinical features.....	342
b. Therapeutic response.....	344
c. Pathology.....	345
d. Arthritic phenomena, subcutaneous nodules and syphilis.....	347
D. Fibrositis and panniculitis.....	348
E. Gouty tophi in adults.....	350
1. Clinical features.....	352
2. Pathologic features.....	356
F. Subcutaneous nodules in periarteritis nodosa.....	357
Summary.....	363
Bibliography.....	367

Clinico-pathologic advances in the study of rheumatic fever during the past three decades have been accompanied by the formulation of a doctrine relating to the specificity of the subcutaneous nodule encountered not only in this disease, but also in analogous conditions. Those who regard the lesion as unequivocal evidence of the "rheumatic state" have advocated, on this basis, the hypothesis that rheumatoid arthritis and rheumatic fever are either very closely allied or actual congeners arising from a single cause. This vexing problem, among others, seems to call for critical review of the data concerning the status of the subcutaneous nodule, with particular reference to its clinico-pathologic significance. It is hoped that a comprehensive discussion will aid in clarifying the existing divergent views and that detailed exposure of the many controversial points will prove useful in stimulating further interest in this broad subject.

HISTORICAL ASPECTS

Many investigators (de Sauvages (1763 (1)); Cbomel (1813 (2)); Froriep (1843 (3)); Jaccoud (1870 (4)) have been credited, at one time or another, with the first description of the rheumatic subcutaneous nodule. It appears, however, from perusal of original works or remarks made by their respective contemporaries that the names of these observers cannot be accorded this honor, as they misunderstood the criteria for the diagnosis of rheumatic fever, or furnished inadequate clinical data, or described conditions unrelated to the rheumatic affection as it is conceived today. In a masterly communication read on April 3, 1810 under the title, "On Rheumatism of the Heart," Wells (5) recorded, for the first time, the occurrence of nodules in patients undoubtedly suffering from rheumatic fever. In his second case he observed that "many of the tendons of the superficial muscles in this patient were studded with numerous small hard tumours, an appearance I have observed only in one other person, a thin and feeble man forty-one years old, who also laboured under rheumatism." In a cogent foot-note attached to the record of this case, Wells remarked: "Dr. Lister informed me that the superficial tendons of Salmon, the subject of the seventh case in this paper, were similarly affected. As Salmon did not mention this to me, and as I did not discover it myself, the same symptom may have existed in several of my patients, labouring under rheumatism, besides those of whom I have spoken." The failure of subsequent writers (Cheadle (6), for example) to credit Wells with the original description of this lesion must be accounted an oversight occasioned by an increasingly voluminous and complex literature that had sprung up in the interim (7). In 1868 Hillier (8) recorded a typical instance of rheumatic fever, characterized by the occurrence of chorea, joint pains, cardiac signs, and subcutaneous nodules; the importance of this observation was somewhat impaired by the interpretation of the lesions as rheumatic "osteoperiostitis." In 1875 Meynert (9) reported a case of subcutaneous nodules in a boy 14 years of age, who was undoubtedly afflicted with rheumatic fever; the conclusions drawn from this observation of a single example were a tribute to his clinical sagacity. He stated that some of the lesions simulating bony exostoses were not truly osseous, but

were, in reality, attached to periosteum. Moreover, he noted their transient course, refusing to credit the prescribed small doses of potassium iodide with their rapid involution. In the French literature the phenomenon has, consequently, received the designation "Meynet's nodules." Three years later, Féréol (10) recorded several cases of ephemeral nodosities in patients alleged to be suffering from "arthritis"; the eruption corresponded closely in its physical attributes with those of angioneurotic edema, a condition that achieved universal recognition only after Quincke's classic description (1882). Troisier and Brocq (1881 (11)) differentiated the rheumatic subcutaneous nodules from the dermatosis described by Féréol, a distinction subsequently accepted by the latter observer. In the same year (1881) Barlow and Warner (12) published the first comprehensive clinical study of these lesions, based on the observation of 27 examples encountered in young subjects. They pointed out the simultaneous appearance of cardiac derangement and suggested that the subcutaneous nodule represented the pathologic equivalent of the rheumatic vegetations on the heart valves. They were probably the first to note the association with erythema marginatum rheumaticum. During this period the clinical concept of the disease was assuming more definite shape. Garrod (13), in his classic monograph, differentiated rheumatoid arthritis from rheumatic articular disease, an important contribution, as both conditions may be featured by the occurrence of subcutaneous lesions. In 1904 Aschoff described the microscopic alterations bearing his name; these structural changes are accepted by most authorities as the only pathognomonic feature of rheumatic fever (14, 15, 16, 17, 18). Up to the last quarter of the nineteenth century casuistic reports of rheumatic subcutaneous nodules emanated solely from Europe. In 1888 Mitchell (19) recorded what was supposed to be the first American case, but perusal of the report reveals insufficient data whereby to classify this instance as an undoubted example of rheumatic fever; the first unquestionable American case was that described by Brown (20). The rheumatic subcutaneous nodule, then considered as a rarity, has now come to be recognized as a fairly frequent phenomenon, even in this country.

OTHER LESIONS FORMERLY CONFOUNDED WITH RHEUMATIC SUBCUTANEOUS NODULES

From a practical as well as an historical point of view, it seems desirable to discuss several types of lesions that have, at one time or another, been confused with true rheumatic subcutaneous nodules. Those of greater importance will receive more detailed consideration.

(a) *Froriep's nodules*: In 1843 Froriep (3) described indurations or "schwielen" observed in the skin, connective tissues, muscles, and bones in 148 out of 150 cases of "rheumatism." It was recognized subsequently that Froriep's conception embraced a heterogeneous collection of obscure conditions unrelated to the rheumatic affection (11, 21). From the available data at hand, there seems to be no resemblance between these vague infiltration and the rheumatic subcutaneous nodules (22).

(b) *Jaccoud's nodules*: In 1871 Jaccoud (4) recorded the occurrence of *cutaneous* nodules in several cases of "articular rheumatism." The lesions were flat or spherical infiltrations, ranging in size from that of a pea to that of a small nut, were variable in number, were often located at a distance from the articulations, and in their physical attributes resembled erythema nodosum, save that they were of smaller size and lacked the bright erythema. Jaccoud related this manifestation to the type described by Froriep, but considered it a rarity. What Jaccoud recorded it is difficult to state; it is possible that he was concerned with cutaneous lesions encountered in examples of chronic bacteremia. It is certain, however, that the lesions described by him differed fundamentally from true rheumatic subcutaneous nodules. The subject will be mentioned again under the caption of dermic nodules.

(c) *Féréol's nodules*: In 1878 Féréol described several cases of ephemeral nodosities appearing on the forehead and backs of the hands of women afflicted with "arthritis," the latter term referring to the occurrence of migraine in two of the three patients. The lesions involved skin and subcutaneous tissues, generally appeared at night, and underwent spontaneous resolution within 24 hours, without leaving any trace. The tumors were elastic in consistency, definitely circumscribed, varied in size from that of a pea to that of a hazel nut

or larger, and were movable with the overlying skin which was unchanged in color. These cases were reported before the subcutaneous nodule of rheumatic fever achieved universal recognition and before the classic works on angioneurotic edema appeared (23, 43, 44). At first they were confused with true rheumatic nodules, though Féréol himself recognized subsequently that they were not identical. It is probable that these lesions represented examples of angioneurotic edema. That this confusion is possible is shown by my recent observation of a case of "infectious arthritis" in a woman 47 years old, in whom there occurred a transient attack of "painful" tumors in the scalp, regarded at first as rheumatic nodules; these lesions were examples of urticaria giving rise to a burning sensation interpreted by the patient as akin to pain. Subsequently, she also had ephemeral swellings of the hands, the entire syndrome being considered as allied to angioneurotic edema.

(d) *Heberden node*. In 1802 Heberden described a condition featured by the appearance of knobs on the terminal joints of the fingers and remarked that "they have nothing in common with gout." These lesions came to be regarded as harbingers of prolonged life and by some as indicating freedom from disease in other joints. Such rules, formulated on the basis of immature rationalization, have many exceptions.

The term Heberden node has been applied to a number of conditions having in common the occurrence of small firm protrusions in the neighborhood of the distal interphalangeal joints. Formerly regarded as an expression of gout, these manifestations are now known to be more commonly observed in association with osteoarthritis or as a degenerative phenomenon independent of articular involvement elsewhere in the body. Occasionally they represent an evidence of gout, as in three examples studied by Brogsitter (226), and, rarely, they may be found in combination with rheumatoid arthritis, tuberculosis of the bones (24), ochronosis (227), and even "juvenile rheumatism" (228). Barker (229) recognized that the Heberden node does not represent a morphologic entity, for the excrescences may be true bony exostoses or may, at times, be caused by softening and flattening of the base of the terminal phalanges or, rarely, may be due to genuine nodule formation, as in gout. According to Wick (24) and others, these lesions are not

encountered in rheumatic fever; in the latter disease subcutaneous nodules may appear over the terminal interphalangeal joints, but neither clinically nor by roentgenogram do these share the attributes of the Heberden node.

The true Heberden nodes seem to have a variable onset and physical attributes. As a rule, they appear insidiously at the lateral aspects of the terminal joints of the fingers, attain the size of a small pea in spurts of growth, and are more disfiguring than harmful. The lesions are usually firm, though in some instances they may begin as soft, translucent, cystic, fluctuant nodes (13, 233) which contain a viscid fluid rich in calcium (233) and which subsequently hardens to form the typical bony protrusion. Generally there are no subjective symptoms; at times the patient may complain of pain in these parts (234). Ankylosis, stiffness or crackling of these areas are not observed (235), though there may be slight deformity of the fingers and, perhaps, a certain embarrassment in their mobility. F. v. Müller (230) reported the occurrence of subluxation of the last phalanges in some cases. The terminal joints of the thumbs often escape (13) and the same seems to be true of the toes (24), but there are occasional exceptions (235). In most cases the lesions are opaque to the roentgen rays; it is, however, possible that this may not hold in the early stages. In any event, the occurrence of areas transparent to the roentgen rays should direct attention to the possibility of gouty deposits.

Wick's thorough study (24) of 100 examples of the manifestation indicates that the vast majority is found in women beyond the menopause, with 56 years as the average age of onset. In 30 cases the knee-joints were simultaneously affected. In 85 per cent of the cases other articulations were involved concurrently, probably in the nature of osteo-arthritis, though the pains were often mild in degree. Rarely, these lesions are observed in younger persons where they may represent evidence of local premature senility. Negroes are said to be free of this manifestation (233).

Charcot's description (231) of the anatomical changes in the nodes appears to show the close similarity of the process to osteo-arthritis, the alterations probably being incident to an aging in these tissues. Brogsitter (226) found more or less severe vascular changes, including obliteration of vessels, which he interpreted as the initiating cause of

nutritional disturbances, leading to osteophytic enlargement of the parts, but it is not clear whether these changes represent an effect of old age or a disease *sui generis*.

(e) *Miliary nodules of Coates and Thomas*: Coates and Thomas recorded the occurrence of miliary nodules of rheumatic origin. The lesions range in size from that of a sago grain to that of a pinhead, are often grouped in small clusters, and are better felt than seen. They are widely distributed in soft tissues as well as over the prominences of the vertebral spines. These observers reported the presence of this lesion in over 25 per cent of presumably healthy children and in practically all instances of childhood rheumatic fever. It was also stated that in its minute anatomy it is the counterpart of the genuine rheumatic subcutaneous nodule. Not only is it alleged to be a common finding in rheumatic fever, but it is also believed to represent an early manifestation of this disease, notably in children free from cardiac abnormalities. The absence of involvement of the heart would appear to call for reconsideration of the significance of the phenomenon. It is true that tiny subcutaneous nodules may be encountered in the course of rheumatic fever (26); this is observed particularly in lesions at their inception or in the final stages of involution. It is probable, also, that the site of a vanished rheumatic nodule may be marked by the presence of pathologic changes recalling the alterations ordinarily seen in this lesion (27). However, the illustrations of sections of a miliary nodule published by Coates seem not to warrant claim for specificity of histologic structure, and it also appears likely that the attempt to seek analogies with the rheumatic subcutaneous nodule is to stretch the point. More recently, Coates classified the manifestation in the group of "fibrositis", the rheumatic etiology of which is dubious (28). Findlay (29) did not encounter this phenomenon in a series of 701 cases of rheumatic fever in children, followed over many years. In approximately 94 cases of children and adults seen in an orthopedic clinic, Sutro (30) observed, and called to my attention, the occurrence of subcutaneous lesions, millet-seed to almond in size, located in the region of the sacro-iliac joints, crest of the iliac bones, and lower lumbar spines; microscopic study showed the nodules to be composed of adipose tissue, without evidence of inflammation. It is apparent, therefore, that great care must be exercised

in identifying subcutaneous lesions, for there are probably numerous varieties of similarly situated structures bearing no relation to the rheumatic affection. My own observations are in conformity with the view expressed by Findlay who was unable to grant the rheumatic origin of the "miliary nodules" as described by Coates and Thomas.

(f) *Osler node*: The clinical importance of this lesion warrants a more detailed consideration. Concrete clinical knowledge of subacute bacterial endocarditis may be said to have been initiated by the Goulstonian Lectures in 1885 when Osler (31) first clearly differentiated ulcerative endocarditis from rheumatic heart disease. In 1908 Osler made another advance in recognizing the prolonged or chronic cases of this condition. The clinical features and the bacteriology of subacute bacterial endocarditis have been elaborated by a number of observers (32-35, 18). Ephemeral tender nodules were described many years ago under a variety of titles by Rapin (36), Heubner (37), Münzer (236) and a few others. The relation of this lesion to subacute bacterial endocarditis was first appreciated by Osler (38) whose classic description of it was based on a study of 7 instances encountered in a group of 10 cases of "chronic infectious endocarditis". To quote from Osler's original paper (1908): "One of the most interesting features of the disease and one to which very little attention has been paid is the occurrence of ephemeral spots of a painful nodular erythema, chiefly in the skin of the hands and feet, the nodosities cutaneae ephemeres of the French. My attention was first called to these in a patient of Dr. Mullen of Hamilton whose description is admirable: 'The spots came out at intervals as small swollen areas, some the size of a pea, others a centimetre and a half in diameter, raised, red, with a whitish point in the center. I have known them to pass away in a few hours but more commonly they last for a day, or even longer. The commonest situation is near the tip of the finger, which may be slightly swollen! . . . They are not beneath but in the skin and they are not unlike an ordinary wheal of urticaria. The pads of the fingers and toes, the thenar and hypothenar eminences, the sides of the fingers and the skin of the lower part of the arm are the most common localities. In one case they were present in the skin of the flank. I have never seen them hemorrhagic, but always erythematous, sometimes of a very vivid pink hue, with a slightly opaque cen-

ter." In December 1908 Osler (39) corrected the impression that this lesion was the equivalent of the ephemeral nodosities of French authors; in this communication he differentiated it from the cutaneous eruption described by Féréol and from the genuine rheumatic subcutaneous nodule. These fundamental distinctions are now recognized.

Further observations in cases of subacute bacterial endocarditis have added to our clinical knowledge of the morphologic variants of the Osler node. The following account is based principally on a study of 42 examples of this lesion found in 102 instances of subacute bacterial endocarditis caused by streptococcus viridans (alpha), the most common etiologic agent of the disease. These observations will be completed by those recorded in other publications.

One of the common symptoms of this condition is the occurrence of transient painful finger tips, the genesis of which is probably analogous to that of the Osler node (237). In occasional instances the subungual region of the affected finger reveals painful linear hemorrhages with surrounding edema (40). At times the digital pulp may be tender, anemic, and show veritable elevation of the soft parts; sometimes such an area may be surrounded by a delicate hyperemic areola (32). When the lesion is erythematous, it corresponds in its essential features with those described by Osler. Libman (32) recorded a number of morphologic variants. Thus, painful erythematous lesions may occur without appreciable elevation of the soft parts; these may range in size from that of a pin-head to that of an entire pad of a digit, and are always tender, particularly in the early stages. Sometimes the affected area is quite bluish or even purple in hue (33, 32). My own observations indicate that a purpuric element is not rarely superimposed (237); the extent of hemorrhage varies from complete and irregular infiltration of the lesion to tiny petechial spots that may be outlined by aid of transillumination, the discolorations standing out as contrasting opaque spots situated in the depth of the skin. It is important to stress that the typical Osler node is a *cutaneous* lesion; the subcutaneous variety will be discussed subsequently.

Osler nodes usually appear in successive crops of a single to as many as 5 or more efflorescences. In many instances fresh lesions occur daily, and it is not uncommon to find nodules in various stages of

evolution. Generally there is a prodromal sensation of itching, tingling, or intense pain and throbbing at the local site, followed by the appearance of the cutaneous lesion within a period of from several hours to one day. The onset is sudden in most cases and, as pointed out by French authors (35), may simulate a panaris, except that complete resolution takes place instead of suppuration and necrosis; hence, the term "false panaris." Lack of suppuration appears to be an outstanding attribute of the Osler node observed in subacute bacterial endocarditis caused by *Streptococcus viridans* (alpha); this characteristic is a replica of the other features of the disease. However, Nichol (41) stated that necrosis may occur occasionally, but furnished no data relative to the etiologic agent concerned in this case. Brill (42) observed necrosis and ulceration in a single instance of influenzal subacute bacterial endocarditis, and suggested that this feature might serve to differentiate it from the variety caused by *Streptococcus viridans*. In Libman's series of 75 examples of the Osler node and in the 42 instances mentioned in this paper, destruction of skin was not encountered.

The sites of predilection are those recorded by Osler (38). Occasionally the heels and instep are affected (37); rarely the ear, nose, and even the oral mucous membrane. In general, the lesions occur predominantly on distal parts of the upper and lower limbs, though at times outlying areas may also be involved. This point is mentioned as there are other forms of chronic bacteremia (with or without endocarditis), which are accompanied by tender erythematous lesions resembling the Osler node closely in its physical attributes, except for atypical localization on the upper extremities (arm, forearm, etc.) and on the trunk. The term Osler node is best restricted to the lesion described by this observer and the variants added by Horder, Libman, and Blumer.

Weber recorded the occurrence of deeper seated nodules that are apt to be found in the palms. It is possible that the clinical characteristics of these lesions may be modified by the thickness of skin in this situation, resulting in the impression of depth; in all other attributes, they are analogous to the Osler node, including simultaneous involvement of true skin. However, there are uncommon instances of subacute bacterial endocarditis associated with genuine subcutane-

ous nodules arising from causes to be discussed shortly; these lesions are to be differentiated from those seen in rheumatic fever. This manifestation generally occurs in the regions of the upper portion of the thighs and in the buttocks. In one instance I observed an isolated tender elliptical nodule situated over the left manibular joint; it was entirely subcutaneous, was fairly movable over subjacent bone, and was not attached to overlying skin which, moreover, was unchanged in color. Complete involution took place in about one week. The case was one of subacute bacterial endocarditis caused by an organism of the diphtheroid group; the nodule could not be examined histologically. A similar lesion situated in the left thigh of another patient revealed, however, the minute anatomical features of a mycotic aneurism of a subcutaneous artery, providing a clue to the probable nature of the first case mentioned. If rupture of the wall occurs in such instances, a hematoma may result, and it appears likely that the large hemorrhages observed on the inner aspect of the thigh by Stahl (238) originated in lesions of the type described.

The duration of the Osler node and its congeners varies from but a few hours to several days or more. When a hemorrhagic component is present, the site of the lesion may remain visible for over a week in the form of a pigmented spot. Tenderness is experienced at the inception, but this symptom usually disappears before the advent of complete involution. Occasionally one sees what appear to be painless nodules on the tips of the fingers, palms, and elsewhere, but careful interrogation of the patient will often reveal that these lesions were originally attended by some degree of pain.

The manifestation has been considered as pathognomonic of subacute bacterial endocarditis (38, 32, 239). There are occasional exceptions encountered in other conditions associated with the appearance of similar lesions. Thus, Libman and Sacks (46) recorded one such case where a nodule was observed in association with atypical verrucous endocarditis and a cutaneous eruption resembling lupus erythematosus. I have also seen the Osler node simulated in two examples of systemic lupus erythematosus (240), in one of which the area was incised owing to a suspicion of suppuration. Blumer (237) encountered the lesion in at least one case of fulminating acute bacterial endocarditis (*staphylococcus aureus*). Several years ago I

observed an example of staphylococcus aureus endocarditis accompanied by Osler nodes on several toes and finger tips; in addition, the palms revealed a few Janeway spots some of which contained minute central pustular puncta. Although the foregoing account has been based on cases of subacute bacterial endocarditis caused by *Streptococcus viridans* (alpha), the Osler node may also be seen in examples of the disease due to other organisms; for example, *B. influenza* (1 case), *B. Diphtheroid* group (2 cases), *enterococcus* (1 case), and *Streptococcus anhemolyticus* (gamma) (1 case). Starling (241) mentioned an example of the node observed by him as a prominent feature in an instance of gonococcal endocarditis. Once I met with an analogous lesion in an example of gonococcal bacteremia; at no time during the course was there definite evidence of endocardial involvement and the patient made an apparently complete recovery. In addition to such cases, there is a peculiar variety of septicemia caused by the gonococcus, in which there are occasionally encountered painful erythematous lesions that may be confounded with the Osler node (Keil) (242). In another publication I have also briefly commented on the relation of the Osler nodes to the cutaneous manifestations observed in cases of chronic bacteremia due to a variety of organisms (75). The clinical specificity of this lesion is enhanced by the simultaneous or successive appearance of flat white-centered petechiae, subungual linear painful hemorrhages, and by such internal manifestations as are commonly considered to be of embolic origin. The Osler node is also encountered in children occasionally, usually those above the age of five, where it presents the same attributes and enjoys the same clinical significance as the lesion in adults. It is probable that the Osler node may appear in cases of subacute bacterial endocarditis complicating congenital heart disease in young children, but thus far I have not met with this combination of events.

When, however, the phenomenon is found in the course of rheumatic fever (47), it seems likely that the case is one of subacute bacterial endocarditis where the patient has not been observed sufficiently or where the causative organism has, for one reason or another, escaped isolation. In reporting the occurrence of subcutaneous nodules in subacute bacterial endocarditis, it is important to exclude mycotic aneurisms of the subcutaneous arteries, lesions that are usually char-

acterized by pain and tenderness, generally show different localizations, and present distinctive histologic attributes. Coates and Coombs (125) observed typical rheumatic subcutaneous nodules with characteristic minute anatomical changes in a case regarded by them as one of subacute bacterial endocarditis, but I must agree with McEwen (243) in the rejection of this instance from the latter category for two reasons: (1) the failure to verify the diagnosis by means of one or more cultures of blood; (2) the astonishing recovery made by this patient, "albeit with severe lesions of the aortic and mitral valves," an observation that can be correlated with the experience of Thayer (259) who pointed out the occasional occurrence of peculiar cases of rheumatic heart disease characterized by clinical features indistinguishable from those of subacute bacterial endocarditis (244). Moreover, it is becoming more evident that there are instances in which both conditions seem to occur simultaneously (15). The precise relation between these diseases is still obscure (260, 261); the weight of evidence, however, appears to indicate that they are separate and distinct entities.

Several interesting points are brought out by study of cases in which the two diseases appear concurrently, the diagnosis of rheumatic fever being based chiefly on the finding of Aschoff bodies in the heart (15): (1) the rarity of a previous history of rheumatic subcutaneous nodules in these patients, although postmortem examinations show that a vast majority of cases of subacute bacterial endocarditis has been afflicted with previous rheumatic heart disease. In a group of 10 children studied by Saphir and Wile, this occurrence was noted in but one instance, and the literature contains no other example of this association. It is possible, as will be shown later, that patients (usually children) exhibiting subcutaneous lesions in rheumatic fever do not reach maturity, except, possibly, in occasional instances. (2) the appearance of rheumatic subcutaneous nodules during the active phase of subacute bacterial endocarditis is even rarer, despite the finding of Aschoff bodies in the heart. This may signify that, under the influence of the former condition, there may be an exacerbation of a smoldering rheumatic infection in the heart (245), but the evidence presented to-date would seem to show that this is a local phenomenon, rather than a general one. If, in the future, examples* of rheumatic

subcutaneous nodules are indeed found in association with undoubted cases of subacute bacterial endocarditis, this will afford satisfactory evidence that active rheumatic disease is concurrently present. No convincing data have thus far been presented in this connection to negate or even to impair the doctrine relating to the genuine rheumatic subcutaneous nodule.

It is remarkable that there are only two reports in the entire literature concerned with the histology of the Osler node (246, 247). Additional interesting data on the minute anatomical appearance of the vessels in the ears have been recorded by Ottander (248). Without discussing the subject in great detail, it appears that the alterations are resident chiefly in the blood vessels in the corium, with prominent changes in the endothelium in the nature of swelling and proliferation of these cells leading to partial or more complete closure of the lumens. It is customary for French authors to speak of "endotheliitis" and to regard these alterations as evidence of a local process rather than one dependent on embolization from the valves of the heart; thus far, attempts to demonstrate the causative organisms in sections of skin have been uniformly unsuccessful. The clinical appearance of the various manifestations seen in subacute bacterial endocarditis probably depends on the size of the affected vessels, intensity of damage to the walls, and on the degree of collateral circulation available; the pathogenesis is, however, still obscure and it is apparent that there is need for further study. Examination of the microphotographs furnished by the above investigators shows that the minute anatomical changes have little in common with those found in the rheumatic subcutaneous nodule, a full description of which will be given in another section of this monograph.

(g) *Janeway spots*: In 1899 Janeway stated (249): "Several times I have noted numerous small hemorrhages with slightly nodular character in the palms . . and in the soles . . , when possibly the arms and legs had but a scanty crop in malignant endocarditis, whereas this has not been my experience with the processes likely to be mistaken for it." In 1906 and in subsequent publications, Libman (250-252) endowed these interesting manifestations with the patronymic title under which they are now known. In 1923 Libman (251) remarked: "In contradistinction to the Osler nodes, these lesions are not tender. The

lesions are of such great value for diagnosis that we have thought it proper that they should have a special designation, and that this designation should carry the name of the eminent clinician who emphasized the importance of them for diagnostic purposes. We have found that some of these lesions may be rather erythematous in character." Although sharply differentiated from the Osler node, the Janeway lesion has often been confounded with it, so that a review of the clinical attributes seems indicated.

This manifestation, occurring chiefly in the palms and soles, but also in adjacent parts of the limbs is featured by the appearance of *painless* reddish or reddish blue macules, sometimes exhibiting a definite hemorrhagic component. The lesions which are usually isolated and few in number, occasionally more numerous, range in size from that of a split-pea to that of a dime piece. At times they may be slightly papular (nodular) to the touch owing to a degree of edematous infiltration, but this is generally uncommon as they are found principally in areas having relatively unyielding skin (palms and soles). They are apt to be more persistent than is the case in the Osler node, but this is sometimes difficult to judge owing to the premature death of the patient from bacteremia. Libman regarded them as practically pathognomonic of acute bacterial endocarditis, though they were seen occasionally in subacute bacterial endocarditis "in a less typical form." In several cases under my observation, I have observed such small spots labeled as Janeway lesions, but in nearly all instances careful questioning of the patient elicited information indicating that they were in all likelihood Osler nodes which in the course of a few days had lost their original painful character. Osler (253) recorded an unusual case of "chronic infectious endocarditis" in which there appeared two peculiar areas of persistent erythema, one on the ulnar aspect of the palm and another on the under surface of the left big toe; these lesions were apparently painless and seem to correspond with the clinical conception of the Janeway spot. These lesions enjoy their greatest usefulness as a clinical sign when they are limited to a few macules situated on the palms or soles.

In a few examples under my observation, the spots on the palms and soles remained macular but were accompanied by efflorescences on other parts of the body; the latter elements showed the attributes

commonly seen in either *Staphylococcus aureus* or *Streptococcus hemolyticus* bacteremia, usually the former. In a few cases the original flat spots on the palms revealed either vesicular or pustular centers, thus permitting the observer to venture a reasonable guess as to the causative organism of the bacteremia. It is probable that efflorescences similar to the Janeway spots may be found occasionally in cases free from bacterial endocarditis; nevertheless, their occurrence in typical form suggests a profound bacteremia in which the possibility of a superimposed endocarditis is great. Such spots are not to be confounded with lesions having a somewhat similar appearance in systemic lupus erythematosus, erythema multiforme exudativum, endemic typhus, and infectious mononucleosis among other conditions.

Libman and Friedberg (252) stated that the Janeway spots are caused by "an inflammatory polynuclear lesion of the walls of capillaries, accompanied by a similar infiltration of neighboring tissue." It is probable that the larger macules, sometimes seen on the dorsum of the feet and around the ankles, may be due to involvement of considerably larger vessels (arterioles), as in one instance under my observation. Further study of the histopathology of these lesions seems indicated in an effort to secure additional data on the pathogenesis of these curious spots.

RHEUMATIC SUBCUTANEOUS NODULES IN CHILDHOOD

A. Introductory remarks

Although the essential attributes of this lesion are fairly distinctive and constant, irrespective of the patient's age, it is advantageous to discuss separately the manifestation as seen in childhood from that in adult life. Such division, though arbitrary, lends itself best to consideration of differential diagnosis, since the latter varies with the age period. There are, in addition, other reasons justifying separation into two groups, the most important of which are the differences in incidence and in degree of clinical specificity.

B. Clinical data

In children rheumatic nodules appear as subcutaneous infiltrations varying in size from that of a split-pea to that of a walnut and, exceptionally, even larger. They are usually spherical or oval in shape

and, as a rule, well circumscribed. Generally, there is more or less projection above the level of the surrounding skin, though in many instances careful palpation may be required to demonstrate the presence of a small localized lesion. The overlying integument is unchanged in appearance and freely movable over the individual nodules. I have not observed inflammatory reactions of the skin, although, according to some investigators (48, 12, 49), slight to moderate redness may be noted occasionally at the inception of rapidly growing lesions. On the other hand, Bronson and Carr (50) stated that this inflammatory reaction does not appear unless there is superimposed trauma or irritation at the site, an observation that can be confirmed. In rare instances (51) purpura may occur about lesions; in one case under my observation, a similar phenomenon was noted in relation to a subcutaneous nodule situated on the foot near the ankle, but the possibility of trauma could not be eliminated with reasonable certainty. In summary, the typical rheumatic nodule is always subcutaneous and is covered by intact skin (52); exceptions are but rarely encountered and in some instances the question of an erroneous diagnosis may arise.¹

The subcutaneous nodules arise from, and are attached to, the deeper structures, such as tendon or tendon sheath, periarticular ligament, superficial aponeurosis, and external layer of periosteum. It appears that muscle tissue may also, though rarely, be involved (53, 51); this I have not observed either in children or adults, but it is conceivable that infiltrations of this sort may spring from overlying connective tissue sheaths. Ordinarily, the nodules are more or less mobile on the subjacent structures mentioned, except when they are derived from periosteum; in the latter case, the firmly attached lesion may simulate a bony exostosis. The nodules are found on the extensor aspects of joints, over flat bony surfaces (skull, scapula, patella, mal-

¹ In several additional examples of rheumatic nodules I have encountered localized areas of redness in the skin directly overlying the subcutaneous lesions (external aspect of one foot, knuckles etc. etc.). That this is probably caused by a mechanical effect, possibly as a result of pressure on small blood vessels, seems to be supported by the following observations: (1) the erythematous areas corresponded to the location of the nodules; (2) the redness of skin was seen at points where the nodules are apt to lie in close apposition to the integument; (3) the erythema disappeared, generally in a few days, as the subcutaneous lesion diminished in size; contrariwise, such fading may be interpreted as indicating that the underlying infiltration is becoming smaller.

leoli), and in the superficially situated tendons and tendon sheaths; more precisely, bony surfaces at points of exposure and tendinous structures as they lie in relation to superficially located articulations appear to be particularly favored. Findlay's statistics (29) based on 73 cases showing this manifestation may serve, in general, to illustrate the sites of predilection; of the 73 cases, the elbow regions were affected in 56 instances, the knees in 37, the ankles in 16, the occipital portion of the skull in 16, the knuckles in 14, the spines of the vertebrae in 4, and the borders of the scapula in 3. In addition to these common sites, nodules may appear in relation to practically any articulation in the body. On the skull the favored parts are the galea aponeurotica over the occipital and parietal protuberances; less commonly the frontal bones; at times, the suture lines of the cranium (13). Symes (54) recorded involvement of the nasal bones and I have notes on one similar instance in an adult. Rarely, the pinna of the ears shows lesions (55, 11). On the trunk the areas affected may be the ribs, sternum, clavicles, scapulae, crests of the iliac bones, spines of the vertebrae, and, perhaps also, the subcutaneous tissue of the abdominal wall (56). The entire surface of the patellae, the malleoli, and the styloid processes of the radius and ulna are other sites occasionally encountered. At times nodules may be found in tendinous structures situated at points distant from joints (personal observation) as well as in the palms; in the latter location, as will be mentioned subsequently, the clinical picture of Dupuytren's contracture may be simulated. Smith and Sutton (57) recorded an unusual case in which the first subcutaneous nodule appeared in the chest wall in an area corresponding to the apex of the heart.

The infiltrations are firm and somewhat elastic in consistency. Sometimes the sensation imparted to the touch is similar to that of a fibroma (58) or of cartilaginous tissue (48, 59) or, less commonly, of bone (60). In their evolution they may become soft and feel like fatty tumors (13). They do not usually cast shadows on roentgenographic plates (61, 62).

The greater the number of lesions the more surely will there be variations in size and the more surely will some attain large dimensions; this is especially true of nodules situated about the patellae, occipital region of the skull, and spines of vertebrae. According to

Swift (53), the larger lesions represent conglomerates of smaller microscopic infiltrations. Pribram (59) pointed out that there is frequently a relation between magnitude and location of nodules. On this basis, for example, he distinguished three varieties: (1) the miliary type arising from tendon or tendon sheath; (2) larger nodules springing particularly from extensor tendons in regions where the latter glide over joints or at points of insertion into articulations; (3) the largest nodules, approximating the size of a walnut, occurring in relation to bony parts lying directly under the integument (patella, malleoli, spinous processes of vertebrae, and skull). Occasionally it may be necessary to make the overlying skin taut in order to render the nodules more evident; this permits the infiltration to shine through as contrasting whitish or grayish areas (52). Simple flexion or extension of joints, causing tension on surrounding skin, will often accomplish this object.

Statistical studies indicate that the average number of lesions observed is about 3 or 4. At times only a single nodule or perhaps 2 may be discovered, but in such instances the utmost circumspection is necessary to avoid errors in diagnosis (29). Occasionally there may be as many as 30 or 40 lesions and, rarely, the number may reach 150 (63) or even 200 or more (6, 55). In some cases the nodules are, from inception, disposed in a remarkably symmetrical arrangement; at times this effect may only be produced after successive crops have appeared. Symmetry of distribution is, however, not an essential attribute.

As a rule, the appearance of subcutaneous nodules, however profuse they may be, is not attended by subjective symptoms, either spontaneous or on palpation (29). Swift (53) attributed the painless character to lack of proximity to nerves. In occasional instances exceptions to this rule are encountered (64); thus, my own observations seem to indicate that lesions situated on the scalp are the ones most apt to produce discomforting symptoms and similar notations have been recorded in the literature. It is believed that the sensation of pain or discomfort, especially likely in rapidly growing nodules, arises from tension on the skin which in this situation is not supported by the usual thickness of intervening fatty tissue. Other investigators have called attention to the occasional occurrence of pain and redness in

lesions located about the patellae where the element of friction is apt to be a complicating factor. Sometimes, particularly in adults, (personal observation) the symptom of pain or discomfort may first direct attention to the presence of this manifestation, otherwise easily overlooked. Horn's belief (65) that the headaches occurring in some rheumatic patients may be caused by infiltrations in the galea aponeurotica is interesting.

One of the principal characteristics of the rheumatic nodule is its occurrence in crops which may be single or numerous. There may be an abundant outbreak composed of lesions of variable dimensions (59). There is a definite tendency to spontaneous involution; in many cases the nodules are truly ephemeral (13), appearing overnight and disappearing rapidly. The manifestation may present itself clinically within a period of from 24 to 48 hours and completely disappear in 3 days (67). On the other hand, Findlay observed lesions apparently remaining over one year and Swift saw examples of the phenomenon requiring months to years to attain complete development. However, such cases are exceptional; persistence of this manifestation is more likely to be caused by numerous repeated crops of lesions, rather than by permanence of individual nodules. According to my own observations, the rheumatic subcutaneous nodules were characterized by fairly rapid course, the average duration being from 4 to 6 days; occasionally they remained visible or palpable for a few weeks, but, in general, any pronounced prolongation of the period of visibility could be explained by the superimposition of fresh crops of lesions. I have observed no example of this manifestation persisting for months or years, whereas this was commonly seen in the nodules appearing in rheumatoid arthritis or syphilis where longevity seemed to be the rule. Complete involution of the rheumatic lesion generally requires more time than the period of evolution; indeed, the nodules have not infrequently attained their maximum size when first appreciable to the senses. The rapidity with which they disappear is variable and no trace is left in their wake. It has been stated that the rheumatic nodule has a tendency to be more persistent in adults than in children, but my observations appear not to substantiate this belief; it is probable that this opinion has arisen, in large part, owing to the inclusion of the subcutaneous nodule of rheumatoid arthritis (q.v.). The appearance

of numerous crops seems to predispose the patient to recurrences in subsequent exacerbations of the disease; for example, it is not uncommon to find the lesions several times in the same patient in subsequent recrudescences of rheumatic fever, though there is often great variability in the course. Thus, French (63) reported an instance in which subcutaneous nodules disappeared in 3 weeks during a first attack of the disease, whereas in a subsequent exacerbation the lesions were profuse and apparently persisted for over 6 months.

There have been many unsuccessful attempts to perform biopsy on rheumatic subcutaneous nodules, this having occurred, also, in one case under my observation. In cutting down on a lesion that clinically appears to be of fairly large size, it is not rare to find that the nodule has apparently vanished. This has given rise to the opinion that the supposed magnitude of a nodule is dependent largely on the presence of surrounding edematous fluid which dissipates itself when the subcutaneous tissues are incised, thus reducing the lesion to the lower limit of gross visibility. It may be useful to transfix the nodule before biopsy is performed. In instances where death seems inevitable, the site of the lesion should be marked out carefully, as it is not uncommon to observe apparent clinical involution of the nodule just before exitus.

It is now recognized that this manifestation occurs far more often in children than in adults. Of the 27 examples of subcutaneous nodules recorded by Barlow and Warner (12), 20 appeared in subjects below the age of 14 years. In a collected group of 115 cases Berkowitz (66) found that 78 patients were under 15 years of age. Although there seems not to be any law governing the occurrence of nodules (64), the frequency in children generally corresponds to the age incidence of rheumatic fever. Thus, the lesions are but rarely seen in infancy and in children below the age of 3 years. Denzer (68) recorded an exceptional case in a child 20 months old. Of 52 instances of rheumatic fever in patients below the age of 5, Poynton noted its occurrence in 8 patients, but in only one case was the child under 3 years of age. It appears that the highest incidence occurs between the ages of 8 and 14, with progressive decrease thereafter. This is in agreement with published statistics (29) showing that the incidence diminishes as the time interval after the initial rheumatic attack becomes longer. The significance of this point will be discussed in a subsequent section.

It appears, also, from statistical studies that the rheumatic subcutaneous nodule is more commonly encountered in England, an observation correlated with the alleged greater virulence of the disease in that country. For example, in 50 consecutive cases of articular rheumatism in children below the age of 12 years, Still (69) observed subcutaneous lesions in 23 patients or nearly 50 per cent. However, in a more representative group of 200 rheumatic children, including examples of chorea and rheumatic heart disease, with or without evidence of articular involvement, Still encountered 55 instances exhibiting nodules (27.5 per cent). This figure, representing an incidence about twice that observed in the United States, applied only to the severe cases requiring hospitalization. For the milder instances seen in an ambulatory dispensary service, Still estimated an incidence of about 10 per cent. Of 87 fatal cases of rheumatic fever in patients below the age of 12 years, Poynton (262) found subcutaneous nodules in 47 instances (54 per cent) and in 8 additional cases their presence was doubtful; this is supposed to be a conservative estimate, as the sites of nodules can occasionally be demonstrated microscopically after death, though they were originally neither seen nor felt during life (67). On the other hand, Findlay (29) observed the manifestation in 10 per cent of 701 cases of rheumatic fever in children, a figure comparable to that recorded by a number of American investigators (Wallace (49), 12 per cent of 124 cases; Ingerman and Wilson (70), 11 per cent of 185 cases; Keil, 14 per cent of 181 cases).² The

² In an interesting paper by Dieuaide (322) rheumatic nodules were reported as having appeared in 14.9 per cent of a group of 141 cases of rheumatic fever observed in Peiping between the years 1921 and 1936. The author denied the rarity of this disease in China and stated that, though the clinical course in his patients seemed mild, "cardiac damage here is as frequent among patients with rheumatic fever, and as serious, as it is anywhere (56 per cent of the whole group had evidence of valvular disease; 22 per cent had congestive failure)." In the two instances that came to postmortem examination, the typical changes of rheumatic heart disease were found. Dieuaide stressed the necessity for accurate observation and necropsy study in localities where rheumatic fever is *reputed* to be absent—a point well taken.

It is also instructive to compare the statistics furnished by American observers in fatal instances of rheumatic heart disease in childhood. For example, Gibson and Denenholz (324) studying 73 cases of the disease in children below the age of 13, in which death occurred and postmortem examinations were made, encountered subcutaneous nodules in 27 patients—an incidence of 37 per cent. Such a high percentage is undoubtedly in part attributable to greater familiarity with these manifestations of rheumatic fever.

of numerous crops seems to predispose the patient to recurrences in subsequent exacerbations of the disease; for example, it is not uncommon to find the lesions several times in the same patient in subsequent recrudescences of rheumatic fever, though there is often great variability in the course. Thus, French (63) reported an instance in which subcutaneous nodules disappeared in 3 weeks during a first attack of the disease, whereas in a subsequent exacerbation the lesions were profuse and apparently persisted for over 6 months.

There have been many unsuccessful attempts to perform biopsy on rheumatic subcutaneous nodules, this having occurred, also, in one case under my observation. In cutting down on a lesion that clinically appears to be of fairly large size, it is not rare to find that the nodule has apparently vanished. This has given rise to the opinion that the supposed magnitude of a nodule is dependent largely on the presence of surrounding edematous fluid which dissipates itself when the subcutaneous tissues are incised, thus reducing the lesion to the lower limit of gross visibility. It may be useful to transfix the nodule before biopsy is performed. In instances where death seems inevitable, the site of the lesion should be marked out carefully, as it is not uncommon to observe apparent clinical involution of the nodule just before exitus.

It is now recognized that this manifestation occurs far more often in children than in adults. Of the 27 examples of subcutaneous nodules recorded by Barlow and Warner (12), 20 appeared in subjects below the age of 14 years. In a collected group of 115 cases Berkowitz (66) found that 78 patients were under 15 years of age. Although there seems not to be any law governing the occurrence of nodules (64), the frequency in children generally corresponds to the age incidence of rheumatic fever. Thus, the lesions are but rarely seen in infancy and in children below the age of 3 years. Denzer (68) recorded an exceptional case in a child 20 months old. Of 52 instances of rheumatic fever in patients below the age of 5, Poynton noted its occurrence in 8 patients, but in only one case was the child under 3 years of age. It appears that the highest incidence occurs between the ages of 8 and 14, with progressive decrease thereafter. This is in agreement with published statistics (29) showing that the incidence diminishes as the time interval after the initial rheumatic attack becomes longer. The significance of this point will be discussed in a subsequent section.

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figures arrived at will vary necessarily with the type of material available for study. Many observers (64, 53) have also pointed out that the incidence differs with the geographic distribution of rheumatic fever and, from time to time, in the same locality. In any event, this manifestation, once considered a rarity in the United States, is now recognized as a fairly frequent phenomenon, notably in children.

C. Evidence for rheumatic nature of subcutaneous nodule in childhood

Coombs (71) regarded the subcutaneous nodule as the most specific manifestation of rheumatic disease. In general, this expresses the doctrine relating to the pathognomonic significance of the lesion, a dictum having its principal field of application in children. On the other hand Hawthorne, who was chiefly concerned with the disease as seen in adults, remarked that "at one time the subcutaneous nodule was considered absolute evidence of rheumatic fever but we know better today." It will be my purpose to show how these divergent opinions arose and in what manner they can best be reconciled.

a. *Heart involvement*: The association of progressive cardiac disease and rheumatic nodules had been noted as far back as 1881 (12). Garrod (13) remarked that "although cases occur in which crops of nodules are developed while the heart shows no evidence of disease, such cases are quite exceptional." Findlay (29) had not observed a single example of this lesion where the heart was not affected. Voelcker (73) had seen but one instance in which there was no evidence of endocarditis or pericarditis, despite an experience covering hundreds of examples of rheumatic subcutaneous nodules. On the other hand, Still (69) stated that he had observed the manifestation accompanied by arthritis and chorea, without implication of the heart; in two children the appearance of nodules was subsequently followed by disappearance of the cardiac murmurs, the heart remaining normal clinically for at least 3 years. Nevertheless, Still remarked: "In some of the few cases in which I have seen rheumatic nodules without evidence of cardiac affection, definite signs of endocarditis have appeared within a few days or weeks after the appearance of the nodules." Merritt (64) encountered but a single instance without associated cardiac lesion, but accompanied by acute articular rheumatism. Smith and Sutton (57) described a case in which chorea was succeeded by subcutaneous nod-

ules in the apparent absence of clinical signs of heart involvement. In discussing the manifestation, Poynton stated that "cases occur in which they precede any cardiac disease, as in the case of a child in whom they developed months before any cardiac lesion and then disappeared, again developing with the advent of a slight chorea. Finally, a mitral lesion of the sclerosing type supervened."

It is likely that the rheumatic subcutaneous nodule is "practically always" accompanied by damage to the heart in one form or another, though this may not be clinically evident at the time of examination. Nearly all of the statistical compilations show that by the time the lesion makes its appearance there is already definite evidence of cardiac derangement. The statement that the heart was normal because murmurs were not audible must be viewed critically, as clinical proof of endocarditis or other abnormalities may only become apparent on subsequent examination. It is probable that some cases would have revealed evidence of myocardial damage had systematic electrocardiographic studies been undertaken; occasionally fluoroscopic examination may reveal the presence of mitral stenosis in the absence of confirmatory *auscultatory* signs. Osler (76) stressed the occurrence of rheumatic valvulitis postmortem in instances where clinical evidence of cardiac murmurs had been lacking. The supervention of a "sclerosing" type of mitral lesion, in the case mentioned by Poynton, would appear to indicate that the valve had, in all likelihood, been affected early in the course, for such change probably requires months and even years to attain this state of development. With one possible exception (74), an atypical example to be detailed in a succeeding section, there appear to be no instances recorded in the literature, where post-mortem examination disclosed complete absence of heart disease in children exhibiting this manifestation accompanied by other members of the rheumatic cycle. My observations seem to coincide with those of Findlay (29) who emphasized that the utmost circumspection is essential to diagnose rheumatic subcutaneous nodule on the basis of a solitary lesion, as he had seen this label applied to lymph nodes in the scalp or an especially pointed condyle. In addition to such common sources of error, disappearing lesions of erythema nodosum and other affections may occasionally also be designated as "rheumatic" nodules (75). In view of his experience, Findlay stated that examples of

"rheumatic" subcutaneous nodules without accompanying manifest signs of cardiac involvement probably belonged in a doubtful category.

Clinical observation has, therefore, revealed the almost invariable association of evidence of organic valvulitis in cases exhibiting genuine rheumatic subcutaneous nodules. Findlay's statistical analysis (29) showed that the phenomenon was encountered twice as frequently in patients with aortic insufficiency (23.7 per cent) as in those with mitral stenosis alone (12 per cent); it was also noted that this manifestation was only found in examples of aortic insufficiency following arthritis or arising spontaneously, but not in the uncommon instances where it succeeded chorea. Bronson and Carr (50) observed that the subcutaneous nodule appears in from 10 to 14 days after the onset of a rheumatic attack, at a time when arthritic signs are subsiding and evidence of cardiac disease is to be expected. Garrod noted the development of, or the intensification of previously existent, murmurs coincident with appearance of nodules. The occurrence of the manifestation during acute exacerbations of the more chronic forms of the rheumatic affection is well known.

Findlay's valuable statistics also show that onset of pericarditis increases the incidence of associated subcutaneous nodules (27.5 per cent). In general, the occurrence of these lesions may be regarded as indicating severity of cardiac damage; this statement is bolstered by statistical evidence showing a disproportionately high incidence of rheumatic nodules in cases manifesting signs of pericarditis or aortic insufficiency (29), "complications" that apparently indicate more extensive and graver cardiac disease. Garrod (13) also stressed the frequent association of nodules and pericarditis which, though it might be subacute in type, was apt to be attended with the most disastrous results. According to Cheadle (6), the larger nodules are to be regarded apprehensively, as they were not only likely to be accompanied by severe endocarditis, but also in many cases by "deathly pericarditis". It is probable that cases showing aortic insufficiency and pericarditis owe their severity, for the most part, to a greater tendency towards superimposed myocardial disease; in any event, the data compiled by Findlay seem to be best interpreted in this light.

b. *Joint involvement*: Findlay found that 90 per cent of patients having rheumatic nodules manifest articular symptoms during some

part of the course; the lesions usually appeared after the onset of involvement of the joints, rarely before it. Fitcher (77) and others noted the rarity of subcutaneous nodules situated about acutely inflamed articulations, a finding with which my observations agree. It is true that this occurrence is less likely to occur in children in whom joint pains are often trifling, but the rule seems also to apply to adults suffering from "acute articular rheumatism". Frequently the subcutaneous nodules appear in from 10 to 14 days after the onset of a rheumatic attack, at a time when the arthritic signs are subsiding. Aside from the points mentioned, it appears that there are no other broad generalizations which may be formulated regarding the relations of these manifestations. Both phenomena may be seen in the same patient and in the same attack of the disease, though they are usually separated by varying intervals of time.

c. *Chorea*: Occasionally one meets with the statement that the association of chorea and rheumatic nodules is uncommon. On the other hand, Davignon (78) remarked that the appearance of subcutaneous lesions indicated a predisposition to the occurrence of chorea. Of 70 collected cases of rheumatic nodules, Berkowitz (66) found chorea in 39 per cent. The concurrent association of both lesions has been recorded by a number of observers (79, 80, 81). They may occur in the same patient in the absence of articular involvement (79). Taken as a whole, the relationship appears to be incidental, as both manifestations are found predominantly in childhood rheumatic fever; however, it seems probable that nodules are not particularly common in "primary" chorea minor, though accurate comparative statistics on this point are lacking.

d. *Torticollis*: Judging from the material available for this study, torticollis appears not to be a common symptom of rheumatic fever. Its association with subcutaneous nodules probably indicates a rheumatic etiology, as in the case mentioned by Brennemann (254). In Weber's case (82), for example, the initial manifestations were arthritis of an ankle and fever, succeeded by torticollis and subcutaneous nodules situated over the elbows, knees, and metacarpo-phalangeal joints. The auscultatory signs revealed by examination of the heart were "not quite decisive". Weber noted that rheumatic torticollis may be confounded with tuberculous caries of the cervical

vertebrae; the appearance of nodules of the type described favored the diagnosis of rheumatic fever. Although there was indefinite evidence of organic valvular disease, the possibility of cardiac involvement cannot be eliminated in this case owing to lack of fluoroscopic and electrocardiographic investigations and the inability to observe the patient for sufficient time.

e. *Salicylate and sulphanilimide therapy*: According to some observers (83), the intake of salicylates influences the rapidity of involution in rheumatic subcutaneous nodules. This is a therapeutic claim that may be viewed critically by those who recognize the vagaries in course, the marked tendency towards spontaneous involution, and the characteristic transiency of the lesions. It is fairly common experience to observe the occurrence of crops of nodules, despite the administration of large doses of the drug (255). In a recent lecture Swift (256) reported that the use of sulphanilimide was likewise unable to prevent the appearance of subcutaneous lesions; this may be of interest in regard to the streptococcal hypothesis of rheumatic fever.

f. *Association with rheumatic erythemas*: I have notes on 4 cases of rheumatic fever in which erythema marginatum rheumaticum and subcutaneous nodules occurred together in the same patient.³ This interesting association was probably first recorded by Barlow and Warner (12) who found it in 7 of their 27 examples of rheumatic subcutaneous nodules, and this feature was later stressed by Cheadle. Since then, numerous instances illustrating this combination have been reported and its clinical significance is now fairly well established. When the two lesions appear concurrently, the rheumatic nature of the case can hardly be doubted. In addition, nodules may occur in association with the circinate erythema described by Bright and by Lehndorff and Leiner; also, with the less characteristic erythema papulatum rheumaticum (257).

g. *Relation to fever*: Many observers (59, 94) have noted that there is often no reciprocal correspondence between the occurrence of rheumatic nodules and the height of the fever curve; a rise in temperature may precede, coincide with, or follow the appearance of subcutaneous lesions in a most irregular fashion. There may be successive crops of

³ Three more examples of this combination have lately come under my observation.

lesions without appreciable fever and, indeed, the temperature may remain normal, despite other evidences of rheumatic activity. In common with other observers, I have seen nodules become clinically visible during convalescence or after the patient had been sent home as apparently cured. The significance of this phenomenon will be discussed in a succeeding paragraph.

h. *Relation to the first attack of rheumatic fever*: It has been said that subcutaneous nodules rarely appear during the first attack of rheumatic fever. The clinical recognition of the initial phases of the disease is extremely difficult (95); numerous postmortem examinations of patients alleged to have been suffering from a first attack of rheumatic fever have shown, time and again, that, in addition to evidences of recent activity, there were also old structural alterations. However, there are occasional examples of subcutaneous nodules appearing in patients who died in the course of an illness interpreted on clinico-pathologic grounds as the original attack of the disease, as in one instance under my observation. It is interesting that this association of phenomena may occur in Porto Ricans who have lived in the United States a short time and in whom a first attack is likely to be correctly diagnosed as such.

i. *Age of the rheumatic infection*: Findlay (29) published interesting statistics on the relation of nodules to the age of the rheumatic infection. Thus, in 53.4 per cent of cases exhibiting these lesions, they appeared within a period of a few months to one year after the onset of what was interpreted as the initial attack of rheumatic fever. The table is reproduced in complete form:

<i>Time after "initial" symptoms</i>	<i>Number of cases of nodules</i>
First year.....	39 (53.4%)
Second year.....	13
Third year.....	5
Fourth year.....	7
Fifth year.....	4
Sixth year.....	4
Eighth year.....	1
Total.....	73

The figures therefore show diminution in incidence of subcutaneous nodules as the rheumatic infection ages. This may explain, in some

measure, the comparative infrequency of the phenomenon in adults, since in a substantial proportion of the latter group the rheumatic process has had its probable inception in childhood.

j. *Relation to convalescence*: Many observers (66, 96) have been impressed by the fact that at times the subcutaneous nodules may first appear as visible lesions, not during a febrile relapse of rheumatic fever, but rather at the tail-end of a recrudescence or even several weeks after the acute phase has apparently subsided, as occurred in several instances under my observation. This phenomenon illustrates the essential chronicity of the rheumatic process, the appearance of the lesions being regarded as evidence of continued activity. The presence of subcutaneous nodules does not necessarily indicate active disease, as they may remain visible weeks, rarely months, after apparent subsidence of disease; more significant, however, in respect to the diagnosis of rheumatic activity is the appearance of fresh nodules, increase in size, or successive crops of lesions (52).

k. *Relation to cardiac failure*: In many instances nodules are observed in patients exhibiting evidence of progressive heart failure. As in the case of the rheumatic erythemas (97), this association of events appears to substantiate the opinion that in many cases cardiac insufficiency is initiated by superimposition of an acute exacerbation of rheumatic fever, rather than by simple mechanical disturbances (98). In other words, the occurrence of subcutaneous nodules is regarded as excellent evidence of rheumatic activity.

l. *Relation to scarlet fever*: There is a variety of "scarlatinal rheumatism" that usually appears several weeks after the onset of scarlet fever or in its period of desquamation. This type is regarded by many observers as of rheumatic origin, because the subsequent course is frequently an exact replica of ordinary rheumatic fever (85, 86). Thus, such cases manifest evidence of endocarditis, pericarditis, chorea, subcutaneous nodules (85, 87, 88) and erythema marginatum (89, 90). These manifestations may also occur following scarlet fever, without being accompanied by articular symptoms. In occasional instances postmortem examination has disclosed the presence of Aschoff bodies in the heart (86); it appears to be accepted by such outstanding authorities as Fahr and Aschoff that scarlatina itself does not give rise to formation of genuine Aschoff bodies (258). Whether

the rheumatic attack in these cases represents the initial manifestations of the disease or a recrudescence of it has not been settled. This fundamental point must be investigated before satisfactory insight into the relation of the two conditions can be obtained. The alliance has, however, not yet been conclusively established, though the evidence seems suggestive; some observers, believing in their close connection, use this point to substantiate the belief that rheumatic fever is caused by *Streptococcus hemolyticus*; others are of the opinion that the association is accidental, being dependent either on the factor of common age groups (93) or on a special predisposition of scarlatinal tonsillitis to favor invasion of the rheumatic agent into the body (29, 258). On the other hand, it seems likely that in some instances the scarlatiniform eruption may represent one of the earliest clinical manifestations of rheumatic fever itself, the dermatosis being non-specific in nature. It is also important to eliminate the factor of drugs as an etiology in the production of scarlatinal rashes, especially when medication is prescribed for the alleviation of the prodromal symptoms of unrecognized rheumatic fever. There seems to be no doubt relative to the existence of cases of true scarlet fever followed by undoubted rheumatic fever, though the precise relationship is at present problematic; what, however, is not admitted by some observers is that all the scarlatiniform eruptions encountered before the onset of rheumatic fever are, indeed, of scarlatinal origin. It is apparent that the subject deserves more intensive investigation.

m. *Relation to polyserositis*: In all likelihood polyserositis represents a heterogeneous syndrome attributable to a variety of etiologic factors; in most instances it is difficult to determine the precise cause, even at postmortem examination. An interesting case of this sort was recorded by Osler (84); this concerned a child 11 years old, observed on three occasions over a period of 19 months. The patient, when first seen, had manifest signs of cardiac failure, requiring abdominal paracentesis. Subsequently, there were recurring attacks of ascites. During the third admission to hospital, subcutaneous nodule appeared on the knuckles, wrists and elbows. Postmortem examination revealed the typical morphologic evidence of polyserositis, with adherent pericardium, enormous hypertrophy and dilatation of the right ventricle, a hypertrophic left ventricle, and the usual signs of

chronic passive congestion of the viscera. The valvular segments were grossly normal. This case was reported in the days before our more precise knowledge of the structural alterations in the heart in rheumatic disease was available, and therefore little attention was paid to the occurrence of Aschoff bodies and interstitial valvulitis etc. The presence of subcutaneous nodules would appear to favor the belief in the rheumatic nature of the case, though, it must be admitted, the evidence was not decisive. The subject of polyserositis bristles with obscurities and warrants more intensive study and, perhaps, reclassification.

D. Prognosis

In general, the appearance of subcutaneous nodules heralds the graver instances of rheumatic fever, though this is variable. The severity of the prognosis roughly parallels the number and magnitude of the lesions. Thus, Cheadle observed 4 deaths in a group of 6 cases exhibiting large and numerous nodules; on this basis he concluded that the occurrence of lesions of unusual size indicated persistent and uncontrollable cardiac disease, progressing almost invariably to fatality. It is undoubtedly true that there are numerous exceptions to these rules (Brennemann, Swift, Keil) and that, on the other hand, many cases run a rapidly fatal course in the absence of such lesions. Findlay (29) presented statistical evidence showing an increased incidence of nodules in patients afflicted with aortic insufficiency or pericarditis, illustrating the principle that involvement of subcutaneous tissue is more apt to occur in more widespread disease. The prognosis in such instances appears to depend not so much on the "complications" mentioned, as on the probability that the myocardium is more likely to be severely affected when the disease becomes more extensive. The weight of evidence seems to favor the view that the heart is practically always involved in one form or another when rheumatic nodules are present, but it is probable that this feature is constant in rheumatic fever itself (99, 14). However, the occurrence of subcutaneous lesions usually signifies a certain degree of severity of cardiac damage. On the other hand, Still stated that he had observed instances in which the clinical course seemed to be unappreciably affected by the appearance of nodules, and I have also seen several cases which apparently support this observation. Swift mentioned instances in which active

physical life was possible years after the involution of the subcutaneous lesions, but this sequence of events is probably uncommon. Cases of rheumatic children exhibiting subcutaneous nodules have been rarely recorded as having lived to maturity; the available statistics, though incomplete on this point, appear to indicate that the average life in these cases is but a short span of years. Of the 26 children whose cases formed the basis of my observations, 13 were followed to their termination. The vast majority of the fatal cases died in from 1 to 2 years after the initial observation of subcutaneous nodules; in one instance the patient came to exitus 8 years later and in still another instance the child lived three years, but died from an intercurrent meningococcal meningitis, an interesting point in the latter case being that there was evidence of activity in the rheumatic process in the heart postmortem. A number of the remaining 13 patients could not be followed adequately; there were, however, 8 examples where a general idea relative to subsequent course could be obtained. Only one child was in good health after a period of 4 years; the other patients had experienced either one or more recrudescences of the disease or were suffering from the effects of myocardial failure.

In one important respect, however, the prognostic outlook has been modified. During the latter part of the nineteenth century, the occurrence of subcutaneous nodules was considered to signify a hopeless prognosis for the immediate attack of the disease. This point of view has been partially modified to the extent that the chances for temporary recovery, at least, are conceded in any individual attack of the condition, though the available statistics appear to indicate that the patient usually succumbs within a limited period of time. It is also true that subcutaneous nodules may appear at irregular intervals in cases of rheumatic fever characterized by a subacute or chronic course (53); it would be instructive to learn what eventually happens to these patients.⁴

⁴Schlesinger's (323) observations on the course of 90 patients seen during the active stage of these nodules are of great importance. His inquiries covered a decade and many of the children had been followed for 5 to 10 years. The mortality of this entire group, which apparently also included persons beyond the age of puberty, was about 40 per cent; about 50 per cent were in good health; and the remainder were alive but ill. He noted that "as quite three-quarters of the deaths took place within 3 years, it is fairly safe to assume that the figures given are a reasonably accurate estimate of the prognosis." The statistics

Finally, Fahr's interesting hypothesis remains to be noted, as it concerns the prognosis of cases featured by nodules. Fahr suggested the possibility that these lesions represent a focus for the rheumatic agent, from which subsequent attacks of the disease may be initiated. It is implied that the "virus" may be harbored in the microscopic infiltrations existing in the subcutaneous tissue and that these may become foci for dissemination of the disease when favorable conditions arise. This interesting hypothesis requires confirmation.

E. Some unusual aspects of the subcutaneous nodule in childhood

1. *Dupuytren-like contracture*: There appears to be a definite clinical picture attached to those rare instances where rheumatic subcutaneous nodules are found in the aponeurosis of the palmaris longus muscle. These cases give rise to the clinical impression of Dupuytren contracture involving principally the third, fourth and fifth fingers of both hands in symmetrical fashion. Pain is experienced on attempting to stretch the affected parts. Palpation reveals bulges or prominences in the depth of the palms owing to the attachment of the nodules to

also indicated that up to the age of 14, children showing nodules stood less than an even chance of recovery (33 deaths: 22 recoveries); and that after puberty recovery was the rule and death uncommon (2 deaths: 22 recoveries). These figures are of interest and are supported to a degree by those furnished in this monograph, but it is apparent that this aspect of the subject has many lacunae. Other significant points mentioned in this paper are: (1) the association of nodules with auricular fibrillation; it is probable that the latter sign may represent a result of rheumatic activity, in association with which the subcutaneous lesions appear; (2) Schlesinger observed two examples of nodules lasting 3 and 5 years respectively; the first child recovered, the second died. It would be interesting to record the clinico-pathologic data (biopsy examination) in such instances, especially if the results of postmortem studies are available; (3) in 5 cases all evidence of cardiac disease disappeared; it is, however, clear that this referred to clinical signs. It is my belief that were such patients to die of an intercurrent ailment at that time, post-mortem examination would reveal ample evidence of cardiac involvement, according to the criteria briefly mentioned in this monograph, provided the case represented an undoubted example of rheumatic fever; (4) an attempt was made to correlate the number of nodules with the severity of the prognosis. Schlesinger recognized, however, that all such rules, which furnish only good general guides, have exceptions.

Schlesinger's valuable report represents one of the first serious attempts to collect data on the further course of patients showing rheumatic nodules. It is an aspect worthy of renewed and extensive investigation for the important light which it may throw on prognosis. It must be emphasized, however, that the material must be carefully collected and doubtful cases be put to one side.

the tendon sheaths. Extension of the fingers clearly demonstrates this anatomical relation as well as the mobility and independence of the overlying skin. Berkowitz (66) emphasized this clinical picture, differentiating it from the true Dupuytren contracture principally because of the lack of adherence of the skin to underlying structures. Moreover, the rheumatic type may regress spontaneously to normal, whereas the genuine Dupuytren contracture is fixed, progressive and apparently incapable of complete involution. Cases of the rheumatic type have been recorded by Scheele (79), Berkowitz (66) and Eckstein (100) among others. I had occasion to observe an instance of this type of contracture in a child 15 years of age, who had undoubtedly suffered from rheumatic fever in early childhood and who was now seen in the inactive phase of the disease; nodules were not demonstrable at the time of examination, nor was there a previous history of the occurrence of such lesions, though it must be admitted that they could have been overlooked in this situation.⁵ Berkowitz also recorded some instances where the fingers could not be flexed in the apparent absence of demonstrable subcutaneous nodules. It seems important to differentiate this manifestation from non-rheumatic deforming arthritis and from the syndrome described by Garrod (101), in which subcutaneous pads were found on the dorsum of the fingers, associated with true Dupuytren contracture in a large proportion of instances (315).

2. *Calcification and ossification:* The occurrence of calcification has been but rarely recorded in microscopic studies on genuine rheumatic subcutaneous nodules (102, 103, 60, 104). The probable explanation will be considered later in the section devoted to a study of the pathologic anatomy. In 1882 Grawitz (102) noted that the lesions examined by him showed varying histologic features, some being composed of fibrous tissue, others of fibro-cartilage, and one nodule (taken from a clavicle!) of a bony-like structure containing calcific deposits; he there-

⁵ In a recent case of rheumatic fever in a girl who manifested the typical evidence of the disease (carditis, erythema marginatum rheumaticum, profuse subcutaneous nodules), there were Dupuytren-like contractures involving the ring and middle fingers bilaterally; the changes were caused by the occurrence of palpably enlarged nodules in the aponeurosis of the corresponding portions of the palmaris longus. The clinical course and the physical findings in this case appear to substantiate the data already described.

fore concluded that these alterations merely represented stages of development in a single process. On the basis of these observations and those reported by Wick (103) and Neuwirth (105), Fahr concluded that calcification may be an end-result in the rheumatic subcutaneous nodule, contrasting with terminal scar-formation in the case of the Aschoff body in the heart. Critical analysis of the recorded data, however, indicates that most of the published examples belong, in reality, to another category of disease. The possibility that calcium may be deposited in rheumatic nodules cannot be accepted without reservation, and it is fair to stress its rarity and, perhaps also, to question the validity of the original diagnosis. For example, Wick's patient (103) showed many features consistent with the belief that the condition was an unusual example of rheumatoid arthritis. A better illustration was furnished by Gräff who recently demonstrated photographs showing calcification in a "rheumatic" subcutaneous lesion, but gave no clinical report of the case. In another publication, however, he remarked that his study had been concerned chiefly with nodules taken from patients afflicted with chronic arthritis, in the absence of involvement of the heart; under such circumstances, the diagnosis of "rheumatic" nodule becomes questionable, especially if one hesitates to accept the unity of rheumatoid arthritis and rheumatic fever. It will be shown subsequently that, though the pathologic features of the subcutaneous nodules in these conditions are somewhat similar, they differ from one another in many respects. For example, the characteristic chronicity of the lesion observed in rheumatoid arthritis is consistent with the occurrence of calcification, whereas this seems unlikely in the case of the genuine rheumatic nodule which usually has a short tenure of life, though the infiltration may perhaps be demonstrable microscopically in some instances after its clinical disappearance. Contrary to the opinions expressed by Fahr (91) and by Sacks (107), and in agreement with that of Steinitz (108), it appears that Neuwirth's widely cited case was an excellent instance of universal calcinosis, as attested by the presence of healed scars in the skin and the location of the calcific nodules about the large articulations; the reasons for the occasional confusion of calcinosis with rheumatic fever have been stressed by Steinitz (108).

Under the special caption of *nodosités à évolution ossiforme*, Roy

(60) assembled examples of periosteal nodules characterized by their unusual fixity, hardness and immobility. In the final stages the lesions acquired the attributes of exostoses, including the property of impermeability to roentgen rays. Roy reported a personal observation and cited the findings of Grawitz (102); although he wished to attract attention to the possibility of the occurrence of this phenomenon, Roy realized that the rarity of proven cases precluded the drawing of broad conclusions. Intimately related to this discussion is the subject of rheumatic periostitis and osteo-periostitis, a concept bristling with many obscurities. It was mentioned that in occasional instances the subcutaneous nodules derived from the external layer of periosteum, particularly on the skull, showed clinical resemblances to exostoses. Hillier (8), Henoch (109), and Hobbs (110) among others had made this error in their earlier descriptions, and it is interesting that Henoch rectified the mistake in a later edition of his book on diseases in children (111). However, there appear to be a few authentic examples of true rheumatic osteoperiostitis (112, 113, 114, 115); practically every case cited concerned children of young adults and in nearly all, the ulnar bones in their upper aspects were affected. With the exception of Virchow's observation (112) based on postmortem examination, the remainder were diagnosed on clinical grounds. It appears that true rheumatic periostitis producing new bone formation belongs to the rarities. Rheumatic "periostitis" without calcification is a frequent phenomenon, most commonly encountered on the skull, and its clinical attributes are explained by the firmness and immobility acquired by nodules in this situation.

3. *Dermic Nodules*: On the basis of Jaccoud's account, Roy admitted a separate category of rheumatic *cutaneous* nodules. It was mentioned that Jaccoud's descriptions were too vague to warrant generalization, and this criticism also holds for other reported cases (116, 58, 117) cited by Roy who realized that the concept was based on tenuous evidence. Thus, Hobbs (116) described a probable example of angioneurotic edema, similar to those reported by Féréal (10). Duckworth's patient was afflicted with syphilis, with little or no evidence of rheumatic fever. There are no recorded examples of this manifestation where histologic studies were pursued. Although in rare instances the rheumatic subcutaneous nodule may become sec-

ondarily adherent to the overlying skin, I have not encountered any example of the lesion arising primarily in the integument and limited to it exclusively. This is the more interesting as the rheumatic process appears to involve collagenous tissue throughout the body. Since the report by Roy, the literature has become practically silent on this point, with the exception of one article to be mentioned shortly, and it seems doubtful that rheumatic fever produces lesions of the sort described in this section. Atypical examples of erythema nodosum must be excluded from this category (75).

In a recent communication Rosenberg (118) recorded two cases of so-called "cutaneous rheumatic nodules" which, though occurring in adults, will be considered at this point for the sake of convenience. Aside from lack of similarity in clinical and pathologic features to the genuine rheumatic subcutaneous nodules, the term "rheumatic" was used in a broad sense inconsistent with the modern concept of rheumatic fever. Definite evidence that these patients were suffering from rheumatic fever was not furnished, and the status of these cases is best considered *sub judice* for the time being. In passing, it may be stressed that erythema papulatum rheumaticum cannot be regarded as a variant of the rheumatic subcutaneous nodule.

F. Differential diagnosis in children

(1) *Subcutaneous nodules in children without evidence of rheumatic (heart) disease*: From time to time instances of subcutaneous nodules have been described as occurring in the absence of evidence of "rheumatic disease". This is of some importance, as doubt is thereby thrown on the specific nature of the manifestation. Critical study of reports of apparent exceptions reveals in practically all cases certain significant differences from the characteristic lesions described in previous sections.

In 1900 Carpenter (119) recorded the occurrence of subcutaneous fibrous nodules in an infant 17 months old, apparently free from "rheumatism". During three months numerous nodules appeared on the extensor tendons over the knuckles, the phalangeal joints of the fingers, including the thumbs, on the right big toe, and on the occipital portion of the scalp. The hands were crippled and deformed. The liver and spleen could just be palpated; there were no other abnormalities.

Subsequently, fresh subcutaneous nodules appeared on the ankles, right knee, external malleoli, big toes, right foot, and back of the right wrist. Microscopic study showed "fibro-nuclear tissue". Later, crops of lesions presented themselves over the ankle joints, patellae, wrists, left palm, and on the ears. The child was observed two years, but at no time was there evidence of cardiac involvement. In 1908 Carpenter (120) recorded another similar example where the subcutaneous nodules were situated on the occiput and left scapula. Microscopic examination showed a fibrous capsule enclosing delicate areolar tissue, richly supplied with cells. Findlay encountered an analogous case but denied the relation to rheumatic fever, noting that the nodules observed in Carpenter's patients had persisted many years, an attribute foreign to the ordinary rheumatic lesions. It must be admitted, however, that the occurrence of crops of nodules in Carpenter's first case suggests the rheumatic variety, though the development of marked deformity of the hands brings to mind the possibility of an unusual instance of rheumatoid arthritis in an infant, a subject to be considered in the immediately following section.

Finally, there is the interesting case recorded by Thompson as an instance of "rheumatic" subcutaneous nodules appearing in the absence of carditis and corroborated by postmortem examination. This patient was 11 years old and had suffered from a condition diagnosed as glandular fever several months previously. He complained of stiffness in the fingers, wrists, shoulders, back and knees, which interfered with running and stooping. On examination he appeared thin and pale. The articulations showed no abnormalities. There was no evidence of other physical lesions. Subsequently, the joints of the fingers of both hands became swollen and "rheumatic" subcutaneous nodules were found over the elbows. When observed later, there was an extensive crop of nodules over the elbows, knees, along the extensor tendons on the back of the hands, and both ulnae. The finger joints were slightly enlarged, but otherwise painless and freely movable. Examination of the heart revealed no abnormal changes. The pulse rate ranged between 88 and 112. Though the nodules became more extensive, no other abnormalities appeared. Until an attack of appendicitis complicated the picture, the temperature reached a peak of only 99°F on several occasions. Following the removal of a diseased

appendix, the child ran a down-hill course, ending in death. It was noted that the subcutaneous lesions disappeared during the post-operative period of about one month. Postmortem examination revealed retro-caecal suppuration, with several metastatic pulmonary abscesses; there was no evidence of cardiac abnormalities on gross and microscopic study. The case was therefore recorded as an unusual example of rheumatic subcutaneous nodules appearing in the absence of involvement of the heart. However, this interpretation may provoke considerable debate, as the features were decidedly atypical; aside from nodules that may also occur in other conditions to be detailed shortly, there was no additional evidence of rheumatic fever. The type of articular disease and the subsequent painless swellings of the finger joints might be interpreted as consistent with some variety of arthritic condition, independent of rheumatic fever. It is known, for example, that cases originally diagnosed as rheumatic fever in childhood, owing to the occurrence of joint pains, may subsequently become transformed into the typical clinical picture of Still's disease or any one of a number of other conditions. In any event, the nature of Thompson's case remains obscure; its rheumatic origin cannot be considered as proven beyond cavil.

(2) *Subcutaneous nodules in Still's disease*: Ever since Still's (121) classic description of the syndrome bearing his name, there has been much debate regarding the status of this disease. Included in the group are cases lacking some of the essential features ("atypical or incomplete Still's disease"). Finally, a corresponding clinical picture has been described in adults (Felty's syndrome) (122).

According to Leichtentritt (123), rheumatic fever, Still's disease, and subacute bacterial endocarditis are related conditions, representing variations in response to *Streptococcus viridans*; the interpretation of his bacteriologic findings have not, however, received universal approbation. Still (121) pointed out the occurrence of localized healed pericarditis postmortem in several examples of the condition. Fahr corroborated this feature in a typical case of the disease, but despite the presence of pericarditis, he denied its rheumatic nature, stating that "especially in the heart, I have not found them (that is, rheumatic granulomata) lacking in a single case (of the rheumatic affection) in children, and I am therefore constrained to impute the

greatest significance to the negative finding in this case." Fahr therefore concluded that Still's disease, as exemplified by characteristic instances of the syndrome, does not properly belong in the rheumatic category. On clinical grounds many investigators regard the condition as a peculiar form of rheumatoid arthritis occurring in children. Organic valvular defect of the heart appear to have been absent in the cases extant in the literature, and the occasional description of postmortem findings also disclose that such alterations as well as interstitial valvulitis and myocardial Aschoff bodies are not found. The so-called endocarditic form of Still's disease is based on indefinite clinical findings and on the inclusion of probable instances of rheumatic fever, for example, Koplik's case (124).

There are a few examples of Still's disease accompanied by the appearance of subcutaneous nodules (125, 126, 127, 128). In addition, there are occasional typical instances of rheumatoid arthritis in children associated with subcutaneous lesions (129, 29). Although in some cases the histologic changes have been interpreted as similar to those seen in true rheumatic nodules, and even regarded as the counterpart of the myocardial Aschoff body (125, 130), the weight of evidence does not seem to support the assumption that etiologic conclusions can be drawn on the basis of such resemblances (see discussion under rheumatoid arthritis in adults). Critical analysis of the recorded cases demonstrates the difficulties in interpreting the significance of the subcutaneous nodules encountered.

The examples reported by Coates and Coombs (125), Babonneix and Lévy (127), and Wilson (128) appear to lack sufficient clinical data regarding the physical attributes of the subcutaneous lesions observed by them. The illustrations published by Coates and Coombs seem not to support the contention that the pathologic alterations really deserve the title of Aschoff body. Debré and Uhry made a histologic examination of a nodule taken from Babonneix and Lévy's patient, but their description of the "rheumatic nodule of Meynet" does not appear to demonstrate conclusively the identity with the "myocardial" Aschoff body; it must, however, be admitted that there were some resemblances, the interpretation of which is largely a matter of opinion. Grenet (131), in discussing the findings of Debré and Uhry, mentioned the longevity of the lesions in that case (1-1/2

years?); it is this unusual persistence in the manifestation that seems to justify its probable separation from the genuine rheumatic nodule. This belief is expressed advisedly, as there are instances where individual rheumatic lesions have apparently been more persistent than usual (Barlow and Warner (12) cited 5 months as the longest period of time), but such examples are uncommon. In Wilson's case (128) no details were given regarding the attributes of the nodules, except that they developed along tendon and bony prominences in the course of 6 months. Koplik's case (124), cited several times in the literature as an illustration of the occurrence of genuine rheumatic nodules in Still's disease, was an undoubted example of rheumatic fever, characterized by the clinical features of recurrent attacks of chorea, polyarthritis, transient crops of subcutaneous nodules in the usual locations, and the development of mitral stenosis. The peculiarity of this case was the presence of articular changes in the hands, accompanied by slight ulnar deviation. Spindle-shaped fingers are occasionally encountered in the course of rheumatic fever (72) and, rarely, the deformities may be even more pronounced (95, 132). Such instances are at times labeled as Still's disease, but the principal features seem to be more consistent with the diagnosis of rheumatic fever stimulating the former condition.

The occurrence of subcutaneous nodules in the course of chronic arthritis in children has been recorded by Findlay (29) and Paterson (129). The descriptions and published illustrations indicate that the subcutaneous manifestations shared in the attributes of the lesions encountered in ordinary adult rheumatoid arthritis (q.v.), for they were unusually persistent and were situated on the free border of the ulna bones near the height of the olecranon processes. The absence of heart disease in these cases, after extended observation, would seem to militate against a belief in their rheumatic origin. Although the examples are too few and the descriptions lacking in many details, it is my impression that the subcutaneous nodules found in Still's disease are similar to those encountered in adult rheumatoid arthritis. In view of the data collected, the occurrence of subcutaneous lesions in this condition cannot be used as absolute evidence of an alliance with rheumatic fever. Further clinico-pathologic investigations would be desirable.

(3) *Gouty tophi in children*: Outstanding authorities on gout (133, 134) have but rarely observed examples of this disease in the first decade of life or cases whose conditions dated back to that period. Of the several instances found in the literature (135, 136), where more or less clinical data were furnished, no mention was made of the occurrence of visible uratic deposits, the diagnoses in these cases having been suggested on the basis of typical gouty paroxysms. On the other hand, the recent literature contains the reports of two extraordinary examples of the disease in childhood, the presence of subcutaneous nodules giving rise, in at least one instance, to the opinion that the condition was allied to the rheumatic affection.

In 1930 v. Schopf (137) reported a case of gout in an infant 5 weeks old. During the fort-night preceding hospitalization, the child was restless, refused food, and vomited occasionally. At this time firm thickenings appeared on the dorsum of the hands, accompanied by stiffness of the fingers. During the period of observation subcutaneous tumors were noted on the dorsum of the hands, fingers, and on the anterior aspect of the right ankle. The lesions were cartilaginous in consistency, partly adherent to the underlying tendons, and covered by skin that was freely movable and normal in all respects. The nodule situated on the right ankle was punctured; aspiration was apparently unsuccessful, but it was noticed that as the needle was withdrawn there exuded a small amount of snow-white material, examination of which disclosed bunches of fine acicular crystals. On the fifth day of illness frank signs of bronchopneumonia became evident. Owing to premature death, metabolic studies could not be undertaken. Postmortem examination revealed gouty deposits in the kidneys, bronchopneumonia, and mucous colitis. Smears from subcutaneous nodules showed typical needles of sodium urate; the murexide test was positive. In this case the tophi appeared in the absence of definite evidence of a gouty articular paroxysm. The report is memorable because the deposition of urates was apparently dependent on endogenous uric acid, the child having been fed on mother's milk which practically corresponds with a purin-free diet. The opinion that, under certain circumstances, human beings are capable of synthesizing uric acid or its derivatives seems to be substantiated by this observation, though the precise mechanism remains obscure.

In 1934 Vining and Thomson (136) recorded an instance of gout in association with aleukemic leukemia in a boy aged 5 years. The patient was admitted with the history of swollen painful ankles of recent date, succeeded in turn by involvement of other articulations. On examination the liver was palpated two fingers' breadth below the costal margin; the spleen could just be felt. Many of the larger and smaller joints were swollen and extremely painful. There was pronounced anemia. The general appearance suggested that of a severe case of Still's disease. Examination of the heart revealed numerous "haemic bruits". There was irregular fever. In the final weeks of illness, multiple subcutaneous nodules appeared about the elbows, wrists and knees; these lesions were regarded as similar to those associated with the "rheumatic state" in childhood. Their presence was interpreted by some observers as indicating a condition of streptococcal origin. Postmortem examination disclosed gouty deposits in the kidneys and leukemic infiltrations of the lymphatic type in the liver and kidneys. Situated about the various articulations were numerous subcutaneous nodules, appearing as chalky deposits of varying sizes. Analysis of material obtained from them showed 64 per cent monosodium urate. An increase of blood uric acid may occasionally be found in leukemia, and it appears that this case probably represents the first where this disease precipitated deposition of urates in a child with an unusual ancestral history of gout.

Finally, it may be noted that, thus far, characteristic tophi have not been seen in the ears during the first decade of life. Under the caption of adult gout, the clinical attributes of gouty deposits will receive more ample consideration.

(4) *Granuloma annulare and erythema elevatum diutinum*: Although instances illustrating these conditions may be encountered in all age groups, the subjects will be considered at this point for convenience. The histologic similarities between granuloma annulare and the rheumatic subcutaneous nodule have been commented upon by few observers. The belief that resemblances in minute anatomy do not necessarily imply etiologic relations is clearly portrayed in this case. On clinico-pathologic grounds many observers have (138, 139) differentiated granuloma annulare from its analogue, erythema elevatum

diutinum, the latter being regarded by some dermatologists as of "rheumatic" origin (140).

(a) *Granuloma annulare*: Subcutaneous nodules have been occasionally recorded in this condition, the lesions being especially situated about the elbows (141, 142). An unusual example of this disease, accompanied by numerous subcutaneous nodules in the scalp, was recently described by Grauer (143). Microscopic examination of skin lesions disclosed areas of coagulation necrosis, surrounded by radiating strands of cells in palisade arrangement; the cellular response in the form of fibroblasts, epithelioid-like cells, and occasional lymphocytes as well as the situation of the pathologic changes in the reticular portion of the corium were characteristic of granuloma annulare. Dissection of a scalp nodule revealed that it was adherent to the pericranium and independent of integument; microscopic study showed numerous foci of coagulation necrosis, with cellular reaction similar to that observed in the skin. The resemblances in minute anatomy of the pericranial nodules to those of rheumatic origin are interesting, but the clinical features of the case clearly indicated absence of rheumatic disease. It has been pointed out that rheumatic fever, peculiarly enough, seems to spare the collagenous bundles in the corium, whereas the foci of coagulation necrosis and surrounding palisade arrangement of fibroblasts, epithelioid-like cells, and lymphocytes are characteristic alterations observed in granuloma annulare. On clinical grounds there seems to be no evidence allying this condition with rheumatic fever.

(b) *Erythema elevatum diutinum*: In occasional instances of this disease there have been recorded previous attacks of "rheumatism" (144, 145, 146, 140). However, on clinical evidence alone, the cases cannot be grouped in the rheumatic category as the term is at present understood, with the possible exception of Bury's case. The opinions of Crocker (138) and of Weidman and Bezancon (140) that the lesions of erythema elevatum diutinum are analogues of rheumatic nodules from the pathologic point of view appear to be based on cursory resemblances. Indeed, even in Crocker's outstanding account of the disease, there was included the case described by Middleton (147), in reality a probable example of rheumatoid arthritis with an unusual

collection of irregular subcutaneous nodules clustered about the fingers. The instances gathered by Hutchinson in elderly persons and considered by him as "gouty" in nature are now recognized as examples of Kaposi's idiopathic hemorrhagic sarcomatosis (148). In typical cases of erythema elevatum diutinum there are found flat, bluish-red, infiltrated, more or less persistent patches situated over the joints, especially those of the small articulations of the hands, elbows, knees and ankles. The condition affects true skin, whereas the rheumatic nodules, though encountered in similar locations, are more transient and fundamentally involve subcutaneous tissue. Aside from the "rheumatic pains" mentioned, there are no other obvious points of similarity to rheumatic fever.

RHEUMATIC SUBCUTANEOUS NODULES IN ADULTS

In their physical attributes and clinical course the rheumatic nodules seen in adults appear to be the counterpart of those found in children (11, 94, 149, 150, 151, 65, 152, 153). Coutts (154) stated that the lesions encountered in older persons were more persistent; this belief was based on impression at a time when the variations in clinical course of nodules were not well known and at a period in medical history when rheumatoid arthritis was not differentiated from rheumatic articular disease. My observations in 9 examples of subcutaneous nodules seen in adults and study of those recorded in connection with undoubted instances of rheumatic fever in older persons appear to reveal no significant differences from the lesions seen in children, except in the matter of incidence and, perhaps also, in the greater tendency towards atypical localization. The literature contains records of several cases presenting recalcitrant nodules (155, 71, 156, 117, 147), but, in all likelihood, these were examples of rheumatoid arthritis in its modern sense. The persistent lesions that have come under my observation were associated either with rheumatoid arthritis or syphilis or some other condition, such as acrodermatitis chronica atrophicans.

Statistical evidence and clinical study indicate that the manifestation occurs less frequently in adults suffering from rheumatic fever (3 per cent of 341 cases) than in children (14 per cent of 181 cases). Notwithstanding these figures, subcutaneous nodules are not rare in the

former group; the low incidence is, in part, attributable to the comparative ease with which the phenomenon may be overlooked. On several occasions, for example, my attention has been drawn to the occurrence of lesions in scalps of women who first became aware of the nodules owing to the discomfort experienced while combing the hair. Garrod (157) and others noted that the lesions are but rarely found after the age of 25 or 30 years. Findlay's statistics (29) show the diminishing incidence of the manifestation as the rheumatic process ages; the material available for this study indicates that the vast majority of adults had their initial attacks many years before and, in a large proportion of cases, in early childhood. Nevertheless, ephemeral nodules and those slightly more persistent may be encountered in rheumatic fever in all age groups.

Of the 9 adults under observation, 8 were women. In 6 of the 8 women the skull was the site of predilection, and in most of the cases the nodules were noted to be tender, a circumstance attributed to the greater opportunity for inflicting trauma during the act of combing. The oldest patient was 36 years of age. In all, there were 3 cases in persons beyond 30 years; the remaining instances were observed in the second and third decades. Although the numbers were too few to warrant conclusions, it appeared that the subcutaneous nodules in adults displayed greater tendency to atypical localization. Thus, lesions were found on the superciliary ridge, frontal portion of the skull, and nasal bones, whereas the regions of the elbows and knees were implicated in but two instances. The manifestation occurred in crops, the individual lesions having a transient course. The average duration of clinical visibility was from 6 to 8 days, though it was sometimes prolonged several weeks. No examples of persistent subcutaneous nodules were encountered in this small series of cases. In one instance lesions were found during each of three acute exacerbations of the disease, a feature more commonly observed in childhood rheumatic fever. It will be seen, therefore, that the essential clinical attributes seem to be comparable to those manifested by subcutaneous nodules found in children, aside from certain relatively unimportant differences. For example, in but one adult case was there widespread generalization of the lesions, a phenomenon seen more often in children. Chorea and erythema marginatum, so frequently coexist-

ent in younger patients, were not observed in this small series of adult cases. In one instance an indefinite eruption appeared simultaneously about the elbows, but its morphology was too vague to warrant precise classification. In another example a widespread erythematopapular rash occurred coincidentally, the dermatosis being considered as due to the ingestion of salicylates.

In practically all the patients there was undoubted evidence of cardiac involvement. In the occasional instance where the signs were at first indefinite, the subsequent course revealed the presence of heart disease. Nearly all the patients had been previously afflicted with rheumatic fever; whenever there was no past history of stigmas attributable to this disease, the physical signs disclosed the presence of well-marked mitral stenosis, a lesion implying a certain chronicity of valvular pathology. In other words, there was evidence of long standing rheumatic disease in nearly all the adults comprising this series.

Relative to prognosis, the limited number of observations precluded drawing of positive conclusions, as there was little opportunity to examine patients over extended periods of time, except in occasional cases. However, no deaths were recorded. In one instance the patient was seen over 6 years after the initial appearance of the subcutaneous nodules, at which time she was found to be suffering from cardiac failure of a progressive type.

DIFFERENTIAL DIAGNOSIS IN ADULTS

A. Subcutaneous nodules in adults without cardiac disease

Cases of rheumatic subcutaneous nodules without apparent simultaneous involvement of the heart have been frequently reported in adults (49). It was previously stated that, so far as children were concerned, practically all examples were accompanied by manifest evidence of cardiac disease; that the incidence of normal hearts was diminishing as methods for detecting abnormalities were being perfected; and that, where such evidence of heart disease might be clinically absent at one time, further observation generally disclosed signs of cardiac involvement. Study of the apparent exceptions to these rules reveals that these cases nearly always concerned adults, an observation consistent with the hypothesis that rheumatic fever affects

the heart less often in older persons. It is, however, my belief that this concept is based on impressions that have not been subjected to close scrutiny, and an attempt will be made to present data along these lines.

Undoubted examples of subcutaneous nodules appearing in the absence of cardiac involvement have been recorded by Hodge (156), Garrod (157), Middleton (147), and others. Yet, it is difficult to accept these cases as bone fide instances of rheumatic fever in the sense in which that disease is conceived today, for in some cases the subcutaneous lesions were decidedly atypical in their attributes; in others, the diagnosis of rheumatic fever was doubtful; and in still others, the observations were too brief to warrant positive conclusions. In the next section the basis for this belief will receive more detailed consideration.

B. Subcutaneous nodules in rheumatoid arthritis

Discussion of subcutaneous nodules in rheumatoid arthritis brings to light many fundamental questions. The alleged relation between rheumatic fever and rheumatoid arthritis has been adduced principally on the basis of: (1) the occasional resemblances in their clinical features, including the occurrence of subcutaneous nodules; (2) the similarities in pathologic changes seen in the tissues about the joints (158) and in the subcutaneous lesions (126, 159); (3) data revealed by immunologic methods, although it appears that this approach is still in the experimental stage.

Some investigators still doubt the specificity of the "myocardial" Aschoff body, but the controversy in that regard seems to be less acute at the present time. The recognition of the typical lesion and its variants has been aided by adoption of morphologic criteria (15, 16). Recently, Aschoff (160) has cautioned against the confusion of vague, non-specific structural changes with those considered to be pathognomonic of the rheumatic affection, criticizing, rather vigorously, the concept of "fibrinoid degeneration or swelling" promulgated by Klinge, and referring particularly to the broad manner in which the hypothesis has been applied. The minute anatomical resemblances between the subcutaneous nodules encountered in rheumatoid arthritis and rheu-

slower, lasting for months and even years in many cases; (3) they were usually painful and tender, with recrudescences of the painful state from time to time; (4) they were not associated with any cardiac lesions; (5) although they might approximate the size of small shot, the nodules frequently measured up to an inch in diameter. With minor exceptions, the points stressed by Pitt appear to be fundamental. In 1912 Hawthorne (72) attempted to show that the differential criteria invoked by Pitt were inadequate; for example, true rheumatic nodules may occasionally occur in adults, may at times approximate the size of a walnut, may be more or less persistent in older persons, and may sometimes be painful. Although there is a measure of truth in Hawthorne's criticisms, on the whole they do not seem to be justified; the authorities cited in support of his contentions described cases in which the diagnosis was either not clear or erroneous. Freund (163) stated that many examples of nodules listed under the caption of rheumatic fever were, in all likelihood, manifestations of primary chronic polyarthritis (rheumatoid arthritis) and the reverse is probably also true. Further, Hawthorne collected examples of subcutaneous lesions in patients free from both rheumatic fever and rheumatoid arthritis, stating that "their occurrence tends to negate the doctrine of the rheumatic significance of subcutaneous fibrous tumour formation. But they also weaken the argument that the occurrence of such tumours in rheumatoid arthritis suggests that this disease is essentially a form of rheumatism."

In 1930 Dawson and Boots (126) made a study of 40 subcutaneous nodules encountered in 200 cases of atrophic arthritis. With but one exception, an instance of Still's disease, the remainder were typical examples of rheumatoid arthritis. Similar lesions were not found in osteo-arthritis. In three cases there was a history of a previous attack of rheumatic fever; in four, there appeared to be evidence of rheumatic heart disease; and in one, the arthritis and nodules seemed to be related to an antecedent attack of scarlet fever. The histologic findings in 14 cases revealed striking resemblances to the microscopic features of rheumatic subcutaneous nodules. In studies of this sort it appears advisable to eliminate, for the time being, instances where there may exist the possibility that one is concerned with rheumatic articular disease, associated with deformed joints (95, 132) or rheumatoid ar-

thrititis superimposed on an antecedent attack of rheumatic fever. Some six years ago, I observed a woman of advanced age, who had been suffering for a long time from cardiac insufficiency secondary to old rheumatic heart disease. There was evidence of myocardial failure, apparently due to mechanical disturbances, an opinion based on the absence of clinical signs of rheumatic activity. Subsequently, she showed manifest evidence of severe diminution in cardiac reserve, and at this time the joints of the hands were sites of chronic atrophic changes. Subcutaneous nodules were found over the olecranon processes near the free border of the bones; the lesions had been present for many months and were characteristic of those observed in rheumatoid arthritis. Death was caused by heart failure, but at no time was there evidence of activity in the rheumatic process. Although a postmortem examination was not made, the clinical features of the case appeared to indicate that the rheumatic process and the subsequent occurrence of rheumatoid arthritis were apparently coincidental and unrelated; the subcutaneous nodules exhibited the attributes of lesions seen in rheumatoid arthritis and had appeared, persisted and even progressed in development, despite apparent inactivity in the rheumatic process.

In 1932 Clawson and Wetherby (159) recorded their findings in a study of 59 subcutaneous nodules encountered in 200 cases of atrophic arthritis. Of the 59 lesions, 48 had occurred in adults 50 years of age or over. The histologic examinations of 20 specimens revealed data similar to those recorded by Dawson and Boots. However, they concluded that the resemblances in histologic structure to that shown by rheumatic nodules indicated a common etiology, probably streptococcal in nature.

In 1933 Dawson (213) summarized his observations in an outstanding contribution, based on a comprehensive investigation of 66 nodules seen in 245 patients with rheumatoid arthritis. This observer stated that in his experience the subcutaneous nodules occurred only in atrophic arthritis and were absent in the hyperplastic variety of the disease. On the basis of histologic examination, he placed the group the nodules in rheumatic fever in a separate category. In the following section

in an effort to determine how far such claims for an alliance between these lesions are valid.

1. *Clinical attributes of the subcutaneous nodules in rheumatoid arthritis*: These lesions are found predominantly in adults in the fourth and fifth decades of life, contrasting with rheumatic fever where nodules are found chiefly in children and young adults; this is in essential agreement with the respective age incidences of these diseases (232). When, at times, this manifestation is seen in adults afflicted with rheumatoid arthritis in the third decade or even before that, it may be attributed to the occasional onset of the condition in younger persons than usual. Rarely, the lesions are encountered in children with deforming arthritis of this type (232). Thus, Findlay recorded an instance in a child 2-1/2 years of age, in whom there were observed two pea-sized nodules over the left elbow. These resembled the lesions found in rheumatic fever, except that they were adherent to the overlying integument. Microscopic examination showed fibrous tissue with foci of necrosis; in one area lime salts were deposited in the necrotic material, with an attempt at osteoid tissue formation. Findlay regarded the deposition of lime salts as a feature foreign to the true *rheumatic nodule*, an opinion with which my observations are in accord. On the other hand, the occurrence of such lesions in persons of advanced age does not necessarily eliminate a rheumatic origin, for recrudescences of rheumatic fever and, more rarely, even initial attacks of the disease may be seen in elderly people. The differentiation will rest on the clinical attributes of the nodules and on the other features characteristic of the individual morbid processes. In rare instances, also, nodules may be found in persons of advanced age, who show evidence of osteo-arthritis, but in such cases it is possible that the two conditions are independent of one another. The weight of evidence indicates that the lesions are found predominantly in rheumatoid arthritis as contrasted with hypertrophic osteo-arthritis. Its incidence in the former disease has been variously estimated as from 25 per cent (213) to only 6 per cent (264).

The nodules in rheumatoid arthritis range in size from .5 cm. to 3.0 cms. in diameter or larger. Sometimes they are scarcely large enough to be palpated, but as a rule they approximate the magnitude

of an olive. The similar lesions in rheumatic fever rarely attain the size of the typical nodules seen in rheumatoid arthritis. The photographs illustrated in the articles by Hawthorne (72), Dawson and Boots (126), and other observers demonstrate the large size of the characteristic tumors in atrophic arthritis; indeed, there is far greater resemblance to the huge fibroid nodes of syphilis (juxta-articular nodules). However, small lesions may also be found in rheumatoid arthritis, but the location, duration, and the associated features will ordinarily serve to differentiate them from those seen in rheumatic fever.

The sites of predilection are given in Dawson's figures (213) based on a study of 245 cases of rheumatoid arthritis. In this group there were 66 patients who exhibited nodules arranged as follows: elbows 60, knees 8, ankles 6, occiput 2, knuckles 6, and spines of the sacral vertebrae 3. The favored area is therefore the region of the olecranon process, but it is important to stress that the lesion is found at a distance of from 3 to 4 cms. below the height of the olecranon, a site generally spared in rheumatic fever. The element of pressure or trauma appears to be of greater importance in this condition than is the case in rheumatic fever. Collins (214), emphasizing the point, noted that the lesions tend to occur at points where bone approaches skin (olecranon, patella, knuckles, scapula, vertebral spines, and tibial malleoli) or in parts exposed to pressure in frail subjects (scapulae and vertebrae). Other areas affected are the height of the olecranon within the bursa (264), forearms, arms (164), thighs (54), and about the finger joints (159). The lesions are usually bilaterally symmetrical, especially those in relation to the elbows and, according to Clawson and Wetherby, this property was illustrated in almost 50 per cent of their group of cases. As a rule, the nodules found near the height of the olecranon processes are distinctive and unlike those seen in rheumatic fever.

The average duration of the nodules in rheumatoid arthritis varies from several months to many years. It is not uncommon to encounter instances where the lesions have persisted for prolonged periods, sometimes as long as 15 years (159). This is practically unknown in the nodules in rheumatic fever, even in adults. On the other hand, there are occasional examples of rheumatoid arthritis accompanied by sub-

cutaneous nodules showing complete involution in a few weeks, at least in a clinical sense; these are usually smaller than the average lesion found in this disease. In some instances the course is relatively acute, with no attendant articular deformities and in such cases one speaks of "acute infectious arthritis" or of "focal arthritis" where a probable focus of entry (tonsils, teeth etc.) seems to be intimately related to the onset of joint symptoms or where the actual removal of such foci leads to temporary or permanent improvement.

As a general rule, the subcutaneous tumors of rheumatoid arthritis appear to be independent of the overlying skin, as in rheumatic fever, but there are occasional instances where definite adherence to the integument has been reported, in the absence of trauma or other complicating factors. In rare cases one or more nodules may ulcerate through the skin at sites of pressure (220). Adherence to the integument is not uncommon in the lesions in rheumatoid arthritis, rare in rheumatic fever.

In many instances the nodules seen in this condition are either spontaneously painful or, more commonly, tender on palpation. This subjective evidence cannot, however, be considered as a differential point, for the same attribute may occasionally be found in the rheumatic lesion. Contrariwise, the nodules in rheumatoid arthritis may appear in the absence of any local discomfort. According to Freund, the lesions taking origin from the periosteum of the olecranon are often spontaneously painful, whereas those found on the height of the olecranon, with or without relation to the bursa, hardly ever produce subjective complaints.

Dawson (213) stated that the lesions occurred, almost without exception, in severe forms of the disease and he regarded their persistence as indicating a poor prognosis. Until more data are collected on variations in the course of this disease, it seems wise to suspend judgment on the relation of the nodules to the eventual outcome. There are many cases exhibiting subcutaneous lesions for protracted periods, occasionally even decades; on the other hand, patients showing the most crippling forms of the disease may be free from nodules.

2. *Pathologic attributes: Introductory comments and scope of discussion:* As the microscopic features shown by the nodules in rheumatoid arthritis and in rheumatic fever are considered by many as closely

allied, if not identical, it will be advantageous to discuss these lesions conjointly. A comprehensive review of the subject reveals that many observers have furnished somewhat variable descriptions of the minute changes; that there is diversity in opinion concerning the alleged resemblances in these alterations to the Aschoff body in the heart; and that the pathologic relation between these subcutaneous nodules and other similar manifestations has been differently regarded. Despite the complexities, certain principles seem to stand out clearly. Pathologists often describe these changes as "rheumatic", leaving to the discretion of the clinician the precise diagnosis. There would be little objection to this procedure, if it were recognized that the unity of rheumatoid arthritis and rheumatic fever is unproven as yet and that the alterations in question may merely represent a type reaction to a variety of noxious agents acting on the subcutaneous tissue (219). Freund and many others have noted that there are examples of nodules recorded under the title of rheumatoid arthritis, though they were, in reality, instances of the lesions seen in rheumatic fever, and vice versa. It is therefore advisable to restrict the study to typical cases of each condition and, with this as the objective, to consider the subject under the following general headings:

(A) The pathologic changes observed in the nodules in rheumatoid arthritis,

(B) The pathologic changes observed in the nodules in rheumatic fever,

(C) The data on the similarities between these lesions and the Aschoff body in the heart,

(D) The significance of certain relatively new methods of investigation as applied to the study of these nodules.

In discussing the minute alterations, emphasis will be placed on the clinical application of the various points mentioned, and a preliminary effort will be made to correlate the anatomical changes with those found in other unrelated conditions.

(A) *Pathology of the nodules in rheumatoid arthritis:* The minute anatomical changes observed in these lesions are highly characteristic, but as will be noted later, they are probably not pathognomonic. Excellent descriptions of them have been given by Dawson and Boots (126), Freund (163), Dawson (213) and Collins (214) among others.

The problem is, however, less concerned with the alterations recorded than with the interpretations and the conclusions drawn from these findings.

The typical nodule consists of several or more smaller units which are visible to the naked eye, but which are variable in size, shape and other attributes. In the well-developed lesion these areas have been described by most observers as composed of three distinct parts: first, an inner or central core of necrobiosis; second, a surrounding zone of large "mononuclear" cells, probably derived from primitive mesenchymal elements and showing a characteristic radial and palisade arrangement; and third, an enclosing zone of dense and relatively avascular fibrous tissue (213).

1. The central area of necrobiosis is apparently caused by a peculiar swelling and disintegration of the collagen bundles coursing through the subcutaneous tissue. This change has been compared with the so-called "fibrinoid degeneration" observed in the heart and other structures in rheumatic fever, an appearance stressed by Klinge and used by this observer as the bulwark of his theory on the pathogenesis of this disease. In a later section an effort will be made to ascertain the pathologic significance of this phenomenon. Briefly stated, there is abundant evidence that this change *per se* cannot be regarded as pathognomonic of rheumatoid arthritis, rheumatic fever, or of "rheumatism" in general. Until the nature of the so-called "fibrinous exudate" is established, it seems wise to designate this appearance as "fibrinoid swelling."

The various staining reactions exhibited by these necrobiotic areas have been studied especially by Dawson who, in particular, noted the fibrillated appearance assumed by the bundles of collagen when the Masson technique of trichrome staining was used. Of greater interest was the demonstration that the reticular framework in this zone is preserved, as indicated by the Laidlaw method of silver impregnation. As the subcutaneous nodule in rheumatic fever shows a similar phenomenon, it has been postulated that the degenerative process is incomplete in "rheumatism", contrasting with the complete necrosis of tissue alleged to occur in gummas and tuberculous caseation (213). However, the evidence on this important differential point is apparently conflicting and, as upon the behavior of the argyrophilic

reticulum a broad hypothesis has been erected classifying the "rheumatic" nodules into one category, it seems pertinent to inquire into the nature of the published data. Among the most important observations cited in support of this view are those recorded by Schosnig (215). It may, however, be noted that (1) perusal of this paper and examination of the microphotographs furnished indicate that this investigator reached only tentative conclusions regarding the practical value of silver stains in distinguishing the necrobiosis of "rheumatic nodules" from the necrosis seen in tuberculosis and syphilis; (2) Schosnig observed that complete necrosis may also be encountered in "rheumatic nodules"—the first exception to the general rule—and then added that this might be expected in old lesions. Yet, in an examination of a nodule removed from a typical example of rheumatoid arthritis (case 11 in Klinge and Grzimek's publication) (216), some of the degenerated areas were completely destroyed as attested by the failure to demonstrate argyrophile fibers, this occurring despite the comparatively recent origin of the lesion—a second exception to the general rule. Likewise, Gräff (104) found that the degeneration in these areas may be practically total, with the elastica fibrils partially or entirely destroyed. Furthermore, Corinini (217), using the Bielschowsky-Maresch technique of silver staining, stated that this method enabled him to differentiate the *incomplete* necrosis seen in gummas from the complete destruction of tissue in tuberculous caseation, for in the former he could demonstrate a normal or increased amount of reticulum framework, in the latter none at all. The studies made by Welti (218) and Kumer and Lang (222) on syphilitic juxta-articular nodes substantiate Corinini's claims.

Dawson and others believe that the phenomenon of necrobiosis of the collagen bundles represents the primary event in the nodule in rheumatoid arthritis. This appearance has been observed in lesions of relatively short duration and such areas may contain infiltrations of small round and large mononuclear cells and, occasionally, considerable numbers of polymorphonuclear leucocytes. Collins (214), basing his opinions on a study of 9 nodules removed from patients with rheumatoid arthritis, took issue with this view and stated that the earliest change is a proliferation of the connective tissue cells of mature and less differentiated type; that this is secondarily followed by "fibrinoid

degeneration" commencing in the avascular center of the new tissue; and that this process advances centrifugally encroaching first on the proliferating connective tissue cells and then on other structures, such as blood vessels and mature collagen bundles. Having accepted the view that "fibrinoid degeneration" appears as the initial event in the subcutaneous lesions in rheumatic fever, Collins used this point to make a basic distinction between the two conditions. We shall see later that the same divergence in opinion exists regarding the nature of the fundamental process in the subcutaneous nodule in rheumatic fever. The weight of evidence, however, appears to support the belief that, irrespective of whether the necrobiosis occurs primarily, secondarily or concurrently with proliferative changes, the pathogenesis of these lesions is analogous, but this conclusion seems to apply only to the subcutaneous tissue. As will be stressed time and again, it seems hazardous to conclude, on the basis of histologic similarities, that these lesions or the diseases causing them are identical or produced by the same agent.

Evidence of necrobiosis may also be found in nodules existing as long as 10 to 15 years or longer. In this respect the lesions appear to differ from the similar, though more transient, rheumatic manifestation, and, on the other hand, to resemble caseous tuberculous tissue in the apparent resistance to organization (214). In long-standing cases, however, secondary degenerative phenomena are often superimposed. Commonly the necrobiotic zone reveals spaces that were previously occupied by cholesterol crystals or other fatty deposits. There may be central softening and liquefaction, with an abortive attempt at absorption of this tissue. When this occurs, the surrounding connective tissue cells may condense into a smooth cellular layer resembling a simple type of bursal or synovial lining (214). Although Dawson did not encounter calcification in these areas, this phenomenon is not rare, as it was found in one instance under my observation and has been reported by a number of other observers (104, 163, 214, 216, 219). In rare cases there may even be an attempt at the formation of osteoid tissue (29). The occurrence of such deposits may be correlated with the attribute of persistence displayed by these lesions; on the other hand, this change is but rarely, if ever, seen in authentic examples of the subcutaneous nodule in rheumatic fever. In a previ-

ous section it was pointed out that the conception of calcification in "rheumatic" nodules appeared to be adduced chiefly on the basis of studies concerned with lesions in rheumatoid arthritis. According to Collins, the larger necrobiotic areas may heal with the production of permanent bulky cicatrices. I have not observed such complete resolution in larger lesions, except when continued trauma caused ulceration with secondary replacement fibrosis. However, this type of involution may occur, considering that spontaneous resolution may take place at least in a clinical sense and presumably as a result of scarring. Information on the nature of the final changes occurring in these lesions is, however, still fragmentary.

2. *Middle cellular layer:* The area immediately surrounding the inner necrobiotic core is composed of an infiltrate showing a characteristic radial distribution and often heaped up in palisade fashion several cells deep, with their long axis perpendicular to the zone of necrobiosis. This creates a distinctive appearance which has been considered by some observers as practically pathognomonic of the nodule in rheumatoid arthritis. The principal component of this reaction has been variously regarded as belonging to the large "mononuclear," epithelioid, or primitive mesenchymal series, whereas others have interpreted it as a more or less differentiated connective tissue cell. McEwen's recent studies (265), based on supravital methods, strongly indicate that the predominant cell is derived from the primitive mesenchymal group. Various straining reactions also reveal the basophilic properties of the cytoplasm of these cells, the presence of nuclei similar to those seen in the chief cellular component of the Aschoff body in the heart, and the occurrence of branching processes such as one sees in reticulum cells or in rapidly proliferating connective tissue cells. A few observers have encountered mitotic figures in this zone (163, 213, 214). Various other cells may also be found, the most interesting being the multinucleated forms which arise either from amitosis or mitosis. These may resemble the giant cells seen in the Aschoff body in the heart and, indeed, Gräff noted their resemblances to the Sternberg or Dorothy Reed cells of Hodgkin's disease. It is said that these large forms are only found in advanced stages of development of the nodules. This appearance, however, is to be expected in primitive mesenchymal elements which rapidly arise by amitosis to form mul-

tinucleated cells, but they are not *per se* pathognomonic of any disease when found in the subcutaneous tissues. In addition, there are variable numbers of scattered lymphocytes and monocytes; in occasional instances large numbers of polymorphonuclear leucocytes and dispersed eosinophiles may be seen. In advanced stages of disintegration, giant cells of the foreign body type may be encountered about fragments of broken-down tissue, apparently an attempt at repair.

When the central necrobiotic area undergoes cystic degeneration, the marginal cellular wall may become hyperplastic and project into the resultant cavity as papillary protrusions (213). The various stages described above illustrate the potentialities for differentiation enjoyed by primitive mesenchymal elements (221).

Regarding the radial and palisade arrangement of cells in these lesions, two questions seem pertinent: (1) Is this appearance always observed in the nodule in rheumatoid arthritis? It is probable that this characteristic morphology may be little evident, especially in the early stages, or may be lacking entirely; for example, note the illustrations published by Crouzon and Bertrand (266), by Gräff (106) and others. (2) Is this structure pathognomonic of rheumatoid arthritis? Here again, it would appear that, while the phenomenon is most often encountered in typical form in this condition, it may be seen in other diseases. It is likely that this appearance represents a general biological reaction to the partial destruction of collagenous tissue found in many affections and that the characteristic morphology is only attained in more or less advanced stages in development. Weil and Delarue (219) described this pathologic response in a variety of "rheumatic" conditions, including gouty tophi. Kumer and Lang (222), making a comparative study of different types of subcutaneous lesions, noted similar alterations in syphilitic, "rheumatic" and several tropical juxta-articular nodes. It is interesting that these changes were exquisitely portrayed in one case which had no syphilis or joint manifestations. Internists seem to be unfamiliar with the analogous *cutaneous* alterations found in granuloma annulare, particularly in advanced stages (223, 224). In this condition, also, the nature of the central necrosis (whether complete or incomplete) and of the surrounding cellular reaction (whether composed of fibroblasts or epithelioid-like cells) has given rise to considerable controversy and, indeed, this

appearance was formerly regarded as of tuberculous origin. In a remarkable example of granuloma annulare in a child, Grauer (143) recorded the occurrence of subcutaneous nodules in the scalp and typical lesions on the skin of the left wrist and right tibia. Inspection of the excellent microphotographs depicting the changes in the nodule in the scalp reveals the striking resemblances to the lesion in rheumatoid arthritis, the radial distribution and palisade formation being exquisitely portrayed. These changes were, moreover, the counterpart of those seen in the true skin (left wrist). The patient showed no evidence of rheumatic fever or of rheumatoid arthritis (225). It has been noted as a point of interest that the integument, though rich in collagen bundles, appears not to be affected in rheumatic fever. It is also only rarely involved in rheumatoid arthritis as an apparent result of secondary encroachment of the lesions in the subcutaneous tissue, notably in areas relatively or absolutely free from panniculus adiposis. It seems fair to conclude from the above evidence that this morphologic reaction, while most often found in rheumatoid arthritis, is not necessarily pathognomonic of that condition.

3. *Peripheral zone:* The cellular area just described is surrounded by a circumferential layer of dense connective tissue. Dawson (213) emphasized the point that this is "responsible for the firm nodular character of the lesion in the gross and accounts for the relative ease with which the nodules may be removed." In the surrounding tissue external to this "capsule" the vessels, notably the arterioles, are commonly the site of alterations similar to those described by Von Glahn and Pappenheimer (267) as pathognomonic of rheumatic fever. In general, these changes are chiefly concerned with "fibrinoid swelling" of the subendothelial layer and, occasionally, of the media, with secondary phenomena such as are commonly seen succeeding such alterations (narrowing of the lumen or small projections into it, depending on the degree of intimal involvement; splitting or more complete destruction of the internal elastic membrane, with replacement by newly formed elastica fibrils in the latter case; sometimes canalization of the intimal layer; and occasionally fibrosis). There is, in addition, a perivascular reaction composed of large and small mononuclear inflammatory cells and proliferation of periadventitial elements resembling the predominant cell found in the middle zone and

sometimes forming a concentric mantle several cells deep. Occasionally the veins may be the site of alterations, affecting the intima chiefly (72). The terminal stages may possibly be represented by the occasional vessels showing thickened walls, with poor demarcation of the various coats (213). These vascular changes seem to be of subacute or more chronic nature, though experiments in animals appear to indicate that alterations of a similar type may be found early in the course. Congested capillaries may also be seen in some instances, but it appears that this does not attain the intensity observed in many examples of nodules in rheumatic fever.

Although the vascular alterations may closely resemble those recorded by Von Glahn and Pappenheimer as pathognomonic of rheumatic fever, other observers have been unwilling to regard these as specific changes. In another publication more complete data will be given concerning the phenomenon of "fibrinoid swelling" in blood vessels, with particular reference to the skin. Here it may be noted simply that the alterations described by Von Glahn and Pappenheimer were encountered in but 10 out of 47 cases of rheumatic heart disease and then as isolated findings in a variety of organs, despite thorough prolonged search; that similar changes have been recorded by others in a number of conditions unrelated to rheumatic fever in its strict sense; and that this type of pathologic response has been considered by some (268) as belonging in the large group of "periarteritis nodosa," this term being used in its broadest pathologic connotation to express a type reaction to a variety of noxious agents.

With rare exceptions, bacterial stains have failed to show the presence of organisms in sections of lesions in rheumatoid arthritis (213, 214, 163, 269). Clawson and Wetherby (159) isolated an "indefinite" type of streptococcus in 12 out of 17 cases of nodules in this disease, the cultures being retained for a period of at least one month. In 1928 Freund was unable to find organisms in such lesions, but three years later, working with Stein (270), he recorded a few positive results (*Streptococcus viridans* and *Streptococcus hemolyticus*). Using a variety of mediums, including anaerobic in some cases, Dawson, Olmstead and Boots (226) obtained sterile results in investigations made on 16 nodules. A critical analysis of this phase of the subject indicates that the work of Dawson, Olmstead and Boots merits the most

serious consideration for the thoroughness with which it was pursued. The consensus of expert opinion inclines to the belief that the positive results are impaired by the use of unreliable methods and the finding of organisms that often appear to be contaminants.

This discussion will be concluded by referring to several clinical phenomena which may be correlated with the nature of the pathologic changes. The large size often attained by the nodules in rheumatoid arthritis may be associated with secondary thinning of the overlying skin in occasional cases, owing to encroachment and subsequent secondary pressure effects (214). In rare instances actual ulceration may take place, especially at points subject to trauma (220). The nodules in rheumatoid arthritis, as will be shown later, exhibit far greater resemblances to the huge syphilitic juxta-articular nodes. The firmness of these lesions may be attributed to (1) the development of a dense avascular "capsule"; (2) the persistence, or relative increase, of the reticulum fibrils found in the central necrobiotic zone; (3) the occurrence of calcification in some instances. There are two other factors that are associated with, or aid in sustaining, longevity: (1) the necrobiotic process which seems to be peculiar in its apparent resistance to organization; (2) the large size of the nodules, which renders absorption more difficult. On the other hand, smaller lesions often disappear in at least a clinical sense, but size alone is probably not the determining factor, for in the analogous tumors of syphilis, huge lesions usually respond to specific therapy, without which they are apt to prove recalcitrant. It is likely that minute necrobiotic areas may occasionally be completely replaced by scar tissue. Three points of fundamental interest remain to be solved; (1) is the occurrence of necrobiosis obligatory?; (2) by what pathologic means do small lesions disappear clinically?; (3) how often does scarring occur in the necrobiotic zones and why is this so relatively uncommon in the larger nodules?

(B) *Pathology of the nodules in rheumatic fever*: A number of observers appear to be agreed in their accounts of the pathogenesis and essential anatomical alterations of the rheumatic subcutaneous nodule (271, 272, 255, 213). The data recorded by MacCallum (272) and by Dawson (213), in particular, would seem to indicate that these lesions do not differ fundamentally from those found in rheumatoid arthritis.

As in the latter condition, the nodules in rheumatic fever are composed of smaller unit areas, grossly visible to the naked eye as millet-seed-sized bodies, and microscopically divisible into the same three zones previously described. The central necrobiotic core, presumed to arise from a primary "fibrinoid swelling" of bundles of collagen, shows similar preservation of the reticulum framework, affording evidence that the degenerative process is incomplete. MacCallum (272) stressed the palisade arrangement of the cells bordering on this inner zone and regarded the morphology of the elements (basophilic cytoplasm, character of the nuclei) as conforming to that exhibited in the Aschoff body in the heart. McEwen (243), using supravital methods of staining, found that the predominant cell in these lesions is probably derived from the primitive mesenchymal tissue.

1. *Inner necrobiotic core:* The evidence thus far presented suggests that "fibrinoid swelling" of collagen bundles apparently represents the primary event in these areas, leading to incomplete disintegration of tissue. Klinge, in several publications, demonstrated microphotographs illustrating the predominance of this change; the palisade arrangement of the surrounding cellular reaction was either lacking (216), in the process of formation, or already well constituted (268). On the other hand, Fahr (273), studying what he considered to be the initial changes in lesions found in tendons, concluded that the primary alteration was a proliferation of connective tissue cells, followed by secondary necrosis—a view corresponding to the one advocated by Collins (214) in respect to rheumatoid arthritis. In these nodules, also, it appears that the palisade arrangement of the surrounding cellular reaction was either inconspicuous or lacking. In a later publication Fahr (91) adopted the view that proliferation of connective tissue cells and necrobiosis may develop concurrently, and there is slowly arising a large body of opinion which tends to accept this view (274, 275, 283).

It is not uncommon, early in the course, to find these degenerated areas invaded by more or less numbers of polymorphonuclear leucocytes. Secondary degenerative phenomena, such as have been described in the section on rheumatoid arthritis, are rarely encountered in the nodules in rheumatic fever, and apparent exceptions to this rule need to be carefully investigated to determine the possibility of an

erroneous diagnosis. In an isolated case Dawson (213) described the occurrence of liquefaction in the inner zone, with proliferation and formation of villous processes in this area; it would be interesting to record the complete clinical data in exceptional instances of this sort. Frank pointed out that when the nuclei in such an area are widely separated and stain poorly, an appearance like that of cartilage may be created. The deposition of calcium in rheumatic lesions in the subcutaneous tissue rarely, if ever, occurs and a review of a few such recorded examples indicates clearly that the clinical features hardly conformed to rheumatic fever. This suggests that the alterations are more transient and therefore more likely to heal completely (by scarring, according to MacCallum (272)) than seems to be the case in rheumatic interstitial valvulitis.

2. *Middle cellular layer:* The views regarding the origin of the cells seen in this zone are similar to those recorded in the corresponding section on rheumatoid arthritis. The basophilic character of the cytoplasm and the occasional occurrence of large cells of this type, mono- and multinucleated, have been noted by some observers, and an effort has been made to identify this pathologic response with that found in the Aschoff body in the heart; more complete data on this important point will be furnished in the succeeding sections. The branching processes in the cell bodies are similar to those seen in primitive mesenchymal cells or in connective tissue cells undergoing rapid hypertrophy, and McEwen's investigations indicate a probable origin from the former source.

When multinucleated forms appear, presumably from amitotic division, the resemblances to the Sternberg or Dorothy Reed cell in Hodgkin's disease become manifest; the probable significance of this will be mentioned later. Some observers (214) have failed to discover mitoses in the infiltrate, an occurrence analogous to that recorded in connection with the Aschoff body in the heart (107). On the other hand, Dawson (213) and MacCallum (272) observed mitotic figures not infrequently, whereas Jacki (166), noting their occasional occurrence, did not believe they were present in sufficient numbers to account for the formation of the many giant cells.

The radial and palisade distribution of the infiltrate in the middle layer, an appearance stressed by MacCallum (272), seems not to be as

nearly characteristic nor as frequent a finding as in the lesions in rheumatoid arthritis. Reference is now being made to the photographs and the descriptions furnished by Fahr (273), Klinge (276), Klinge and Grzimek (216), Merritt (64), Collins (213), Findlay (29) and others. Merritt's case is of interest as it concerned a nodule of unusually long duration. In most instances the pathologic appearance is that of an incipient attempt at radial and palisade grouping, but there are a few instances where this effect seems to have been definitely achieved (213, 268).

3. *Peripheral zone:* In the rheumatic subcutaneous nodule the presence of a well developed "capsule" is apparently not observed and this partly accounts for the difficulties met with in removing such tumors for study. The more rapid course of these lesions may possibly explain the failure to form a firm connective tissue envelope, but even in nodules of longer duration this appearance is not as distinctive as in rheumatoid arthritis. In this general area and in the adjacent external parts, the vascular and edematous elements are striking and probably more conspicuous than in the case of rheumatoid arthritis. The myxomatous character of the lesion has been stressed by a few observers. The intense edema of these parts renders the indistinct boundaries of these nodules even more tenuous, and it is common to find, in excising such lesions, that they have apparently vanished. This seems to be caused by the dissipation of edematous fluid during the process of removing the tumor, thus reducing the size of the lesion to the original microscopic dimensions of the infiltrate and the central necrobiotic core. Corresponding to this edematous element, the number of capillaries is generally markedly increased (125), and it may be so intense that the appearance of renal tubules is simulated, as in one case studied by Collins (214). This neo-formation of capillaries seems more striking than the changes in the arterioles (277), which may also be affected in a manner similar to that seen in the nodules in rheumatoid arthritis (107, 213). In occasional instances thrombosis of vessels has been noted (29, 107). With rupture of small vessels, foci of hemorrhage may be caused at times (262, 278). The increased vascularity and the attendant banal cellular reaction may produce an appearance not unlike that of granulation tissue (29).

The data recorded thus far permit one to inquire about the curious feature of rapid clinical disappearance in a few days, so commonly observed in these lesions. It is possible that the necrobiotic process may be reversible in the sense of speedy absorption of the disintegrated material; yet, observation of such lesions at postmortem (279) after apparent disappearance seems to speak against this view. It appears more likely that in certain cases the necrobiotic process is altogether absent or minimal in extent and that with the absorption of the vascular and edematous elements, the site is no longer appreciable to the senses. The study of incipient and transient nodules will provide more data to explain this curious and fundamental behavior which is so common in the lesions in rheumatic fever and less often seen in the smaller nodules in rheumatoid arthritis.

Having discussed the similarities between the subcutaneous nodules in rheumatic fever and in rheumatoid arthritis, their dissimilarities may now be summarized. In rheumatic fever it is the rule to find that: (1) the focal areas of necrobiosis are not nearly as apparent (214), though exceptions do occur; (2) the necrobiotic core is but rarely the seat of secondary degenerative changes, even when the lesions are of relatively long duration; liquefaction and its succeeding phenomena appear to be uncommon and the occurrence of calcification seems doubtful; (3) the necrobiotic zone apparently does not manifest the same degree of resistance to organization displayed by lesions in rheumatoid arthritis; (4) the radial and palisade distribution of the middle cellular layer is not as well developed as in the average example of rheumatoid arthritis, though in some cases this appearance is roughly or, more rarely, completely achieved; (5) the fibrotic "capsular" effect is much less evident; (6) the edematous element is more pronounced, rendering the boundaries between healthy and diseased tissue more indistinct and causing difficulties in removing the lesion for study; (7) the vascular element more nearly resembles ordinary granulation tissue, with numerous newly-formed capillaries; arteriolar changes are, however, analogous to those found in rheumatoid arthritis, though usually less pronounced. Whether the points mentioned are fundamental or whether they merely express variations in intensity of damage to the same tissue, it is at present difficult to state. There is, however, adequate reason to believe that the reac-

tions displayed by the subcutaneous bundles of collagen are not specific, much as happens in the case of the panniculus adiposus (28), and that the recognition of the various types of nodules will rest chiefly on the clinical features, therapeutic responses, and the discovery of "revealing" substances, such as monosodium urate etc.

Finally, there remains to be considered the bacteriologic findings in the nodules in rheumatic fever. It appears that the claims for a streptococcal etiology began with the investigations pursued by Poynton and Paine (1900) (280) who cultivated a gram-positive diplococcus from several nodules. These results were chiefly obtained in post-mortem bacteriologic studies, the pitfalls of which are better known today. In any event, subsequent observers reported variable findings, some positive for the "streptococcus" (281), others completely sterile (94, 29, 163). Nearly all the attempts to demonstrate the causative organism in sections of tissue have been unsuccessful. In passing, Costa's (282) paper, widely quoted in this connection, is in reality not concerned with this problem, for (1) he cultivated a *gram-negative* diplococcus from an unusual lesion in an unusual location (left subclavicular region); (2) in the communication immediately following this paper, it is evident that he used the term articular rheumatism in its broadest sense. At the present time it may be said that bacteriologic investigations have been fruitless in solving this vexing problem and that the weight of evidence is against the assumption of a primary deposit of any type of streptococcus as the initiating cause of the rheumatic nodule.

(C) *Comparisons with the Aschoff body in the heart:* The close association of the rheumatic subcutaneous nodule with evidence of cardiac involvement has naturally led observers, ever since the publication of the paper by Barlow and Warner, to seek pathologic resemblances between these lesions and the changes in the valves of the heart; subsequently such comparisons were extended to the Aschoff body (271, 272, 283, 284). Thus, Sacks (107) stated: "Pathologists are generally agreed upon the fundamental histologic similarity between the rheumatic subcutaneous nodule and the Aschoff bodies." According to Fahr (91), the dissimilarities could be attributed to the varying responses of different tissues to the same etiologic agent and, perhaps also, to the different stages in evolution studied at the time.

Critical analysis of many reports in the literature and study of the published photographs appear to reveal that the resemblances were adduced, in great part, on the basis of analogy. In support of this opinion Saphir and Wile (15) stated: "From our experience, however, we have come to the conclusion that the Aschoff body found in the heart . . . and the rheumatic nodules found in the subcutaneous tissues are morphologically different structures. The latter is a non-specific tissue inflammation somewhat resembling Aschoff bodies, the former . . . is more likely a specific tissue reaction." Gross and Ehrlich (16), in agreement with this belief, remarked that "in some tissues, viz., subcutaneous nodules, tendon sheaths, and so on, legitimate doubt exists concerning the identity of these lesions with those found in the interstitial tissue of the myocardium." It will be my endeavor to outline the likenesses and the dissimilarities between these lesions.

(a) The resemblances between the rheumatic subcutaneous nodules and the Aschoff body in the heart seem considerable. (1) In both there may occur "fibrinoid swelling" in the collagen bundles, with preservation of the reticulum fibrils; (2) the chief cells in each lesion show similar appearances (basophilic cytoplasm, structure of nuclei, positive pyronin reaction etc.), indicating a probable origin from similar tissue; (3) the vascular changes found in some subcutaneous nodules are analogous to those described in rheumatic fever by Von Glahn and Pappenheimer (260); (4) supravital methods of study (McEwen) show that the predominant cells in the rheumatic subcutaneous nodule are derived from the primitive mesenchymal tissue chiefly, and a similar view is held by most observers regarding the principal component of the Aschoff body; the data on this method and the relation to the findings in the nodules in rheumatoid arthritis will be discussed at the conclusion of this section. Each of the first three points mentioned requires more detailed discussion in an effort to show how far we may regard the changes as significant for the recognition of rheumatic fever.

1. The phenomenon of "fibrinoid swelling" in rheumatic fever was noted many years ago by Geipel, Fraenkel and others. In recent times this finding has been given prominence by Klinge and used by this observer for the identification of the "rheumatic state" and as

an evidence of "hyperergic inflammation" in the sense of Rössle (285). Discarding Neumann's (286, 287) conception of a true fibrinous degeneration of connective tissue, comparable to amyloid degeneration, Klinge came to regard this appearance as due to swelling of the ground substance (221, 288) in which the bundles of collagen are embedded. Because the areas partially take the fibrin stain (214), there are some who believe that true inflammatory fibrin may actually be deposited, although the specificity of such staining methods is open to considerable doubt. Still others attribute the change simply to varying degrees of edematous infiltration of the collagen bundles or of the intervening intercellular substance, and some consider the process reversible, especially in the early stages (284). In any event, the precise genesis of the phenomenon is still obscure and the present custom is to designate it as "fibrinoid swelling." According to Klinge, this represents the earliest lesion in the rheumatic process, antedating the appearance of the characteristic cells forming the typical Aschoff body in the heart. However, it has been pointed out by Gross and Ehrlich (16), and confirmed by Gräff (284), that the stage of fibrinoid swelling of the interstitial tissue in the heart, taken by itself, does not present a structure pathognomonic of rheumatic fever; only the well-developed Aschoff body and its variants are considered as the specific changes in this disease. In a similar vein Aschoff (1934) (274) took issue with Klinge's hypothesis; he stated that the nodules in the heart may arise independently of "fibrinoid degeneration" and that this could be demonstrated especially in fresh cases of rheumatic fever in children dying in a first attack of the disease. More recently Aschoff (275) concluded, on the basis of an analysis of experimental work, that either pure proliferation or fibrinoid swelling may occur as the primary event, depending on the severity of damage to the tissues. This, in a modified form, represents an elaboration of the old view advocated by Fraenkel and taken up by Fahr, that both types of change may appear concurrently as a reaction to the rheumatic "virus." In brief, then, Aschoff does not regard the stage of fibrinoid swelling as an essential precursor of the typical nodule in the heart, and it remains to be determined how far this view is also true of the lesion produced in the subcutaneous tissues.

It is now also recognized that this degenerative appearance may occur in a variety of conditions, clinical and experimental, bacterial and non-bacterial (284, 289). In late years, therefore, the significance of this finding has suffered considerable depreciation, not only in respect to the conception of rheumatic fever, but also as an evidence of "allergic inflammation" (275b). In several recent publications Aschoff and his pupils have criticized, rather severely, the entire hypothesis, referring particularly to the broad manner in which it has been applied to conditions obtaining in the human being. To these objections similar criticisms may be applied to Klinge's analysis (276) of the nature of certain cutaneous lesions, such as erythema multiforme exudativum (Hebra), erythema nodosum etc., which have been loosely classified in the category of "rheumatism" on assumptions of tenuous character. Rössle's conception (285) of "hyperergic inflammation," the fundamental basis of Klinge's views, merits interest as an hypothesis that has stimulated a vast amount of experimentation in animals, but it remains, nevertheless, an hypothesis founded on a heterogeneous collection of "rheumatic" conditions. As stated in the words of Aschoff (290), "no clinician or pathologist would allocate to the picture of specific infectious rheumatism the recorded cases of Rössle." As in the case of the "chronic granuloma," it is likely that this obscure group will be subdivided into clinical entities as time goes on. In like manner, the conclusions obtained along these lines in experiments on animals require the utmost circumspection in applying them to diseases in human beings. Such controversies are apt to arise when pathologic observations are not rigidly controlled by clinical knowledge. No one can deny the value of such interesting work but it seems well, as has been repeatedly stressed by Aschoff, to exercise great caution in framing broad generalizations, without subjecting them to the ultimate critique of clinical observation.

2. The vascular changes described by Von Glahn and Pappenheimer as pathognomonic of rheumatic fever have been mentioned briefly in a previous section. Karsner and Bayless (291), studying the alterations in the coronary arteries, concluded that, though the appearance of "fibrinoid swelling" was nearly always observed in these vessels in rheumatic fever, its occurrence is, by no means, pathognomonic of the disease. That similar or even identical changes in the blood vessels may be found in a wide variety of diseases in human beings and in

animals subjected to various influences seems definite, especially if the same care is taken in making the pathologic examination as was made by Von Glahn and Pappenheimer. It appears unlikely that these vascular lesions, though often encountered in rheumatic fever, can be interpreted as pathognomonic of that disease in its restricted sense. This is especially true if one interprets these changes as belonging in the broad group of "periarteritis nodosa," (268) rendering the latter conception even more inclusive than it has already become.

3. Cellular aggregates highly suspicious of the Aschoff body may be encountered in subcutaneous nodules (292). This is, however, also true of various other such lesions in which the mesenchymal tissue is affected to the point of rapid proliferation. Weil and Delarue (219) observed such an appearance at the periphery of various types of nodules and stated that the Aschoff body may be simulated "in conditions other than the *maladie de Bouillaud*." They also concluded that the sequence of degenerative and proliferative changes represents a general biologic reaction to a variety of noxious agents, including gout. Although the evidence on this point seems clear, it must be admitted that there are close morphologic similarities between the Aschoff body and the infiltration in the galea aponeurotica (Jacki, Tilp) (293).⁶ How far supravital studies will aid in resolving this vexing problem will be discussed in a succeeding section.

(b) On the other hand, there are observers who have been unwilling to identify the lesions found in the subcutaneous tissue with those in the heart in rheumatic fever. In support of this view, the following evidence has been cited:

1. The subcutaneous nodule is composed of small units which are, however, grossly visible; the Aschoff body in the heart is a microscopic structure. Occasionally the latter may be macroscopic (107, 272), but the weight of evidence inclines to Fahr's belief that this is a rare occurrence and that in most cases scars in the myocardium created a simulating appearance.

2. Degenerative phenomena in the form of necrobiosis of collagen bundles is a far more prominent feature in the subcutaneous nodule than in the Aschoff body in the heart.

⁶Note the similar changes in the nodules in the scalp of Grauer's case of *granuloma annulare* (143).

3. The subcutaneous lesion is regarded as fundamentally exudative, that in the heart as essentially proliferative.

4. In the Aschoff body in the heart the pyronin reaction is marked (91) owing to the occurrence of large and often multinucleated cells derived from the mesenchymal series; this reaction is said to be weaker in the subcutaneous nodule because of the lesser incidence of these cells, but there are probably occasional exceptions.

5. The Aschoff body heals by scar formation, whereas the rheumatic subcutaneous nodule is said to terminate often by calcification (91). The evidence on the latter point is, however, doubtful and apparently contradicted by the data collected in this monograph.

It is possible that these differences may not be fundamental in a pathologic sense, and observers have attempted to reconcile them by assuming variability in tissue response (91) or variations in the stage of development (166, 294). Whatever view is held, there is insufficient evidence, at present, to uphold either hypothesis, except presumptively, and the problem requires solution by other means. It is fair to note that Gräff (284) regards the changes in the tendon nodules as showing a higher degree of histologic specificity than the Aschoff body in the heart, but it is a view which requires better proof than has been furnished. It seems more probable that the true rheumatic subcutaneous nodule has no incontrovertible claim to specificity by virtue of the histo-pathologic alterations, with the possible exception of the infiltrations in the galea aponeurotica (293), but that its recognition will be based on clinical criteria as applied to the various types of subcutaneous lesions studied and to the causative diseases themselves. The data presented in this monograph can be interpreted as supporting this principle. It appears that the heart acts as a "noble organ" vulnerable, however, to rheumatic fever, whereas the subcutaneous tissue shows much less discrimination towards a variety of noxious agents.

(D) *Experimental studies*: Two relatively new methods of investigation have been used recently for the purpose of throwing light on the nature and pathogenesis of the rheumatic nodules. The interesting results obtained warrant a detailed consideration.

1. *Supravital studies*: In 1926 B. Sacks (107), concluding that the subcutaneous nodule is the homologue of the Aschoff body in the heart,

suggested that the former he investigated by supravital methods in an effort to ascertain the nature of the cellular reaction in the latter structure. In 1932 McEwen (243) examined supravitaly the scrapings of 11 rheumatic subcutaneous nodules, by exposing them to weak solutions of neutral red and Janus green. As control material, the skin and subcutaneous tissue from human beings and rabbits were studied in a variety of conditions. McEwen found that the predominant cell in the rheumatic nodule was derived from the undifferentiated mesenchymal series of the loose connective tissue and, probably also, from "desquamated endothelium". It appeared in the form of oval mononucleated or larger multinucleated cells, showing transitions to other spindle-shaped elements. These types displayed but little evidence of phagocytic power and their failure to react to neutral red distinguished them from epithelioid cells, monocytes, and plasmatocytes. The latter two varieties were also, though rarely, encountered in the smears of the rheumatic nodules. The spindle-shaped elements suggested the presence of fibrocytes but were regarded as a more undifferentiated type of connective tissue cell.

In observations to be published shortly, McEwen (1938) (265) extended these investigations to include 8 nodules taken from patients with rheumatoid arthritis, 2 syphilitic juxtaarticular nodes, 1 gouty tophus, several sebaceous cysts and specimens of the subcutaneous tissue from human beings and rabbits. Two of the 8 nodules in rheumatoid arthritis showed cells qualitatively and quantitatively identical with those of orthodox rheumatic nodules. In the remaining 6, the findings were similar, though there were some differences that were regarded by McEwen as minor. In 2 such lesions, for example, he encountered minute pale bodies staining with neutral red, an appearance which he believed could be easily differentiated from the more conspicuous bodies in the 2 syphilitic nodes examined by him. Another interesting finding was the higher incidence of spindle-shaped elements found in 4 nodules in rheumatoid arthritis, an observation consistent with the greater tendency for such lesions to develop a "capsule". Of importance was the occurrence, in 5 cases, of multinucleated cells which were identical with those previously described in the nodules in rheumatic fever. On the whole, then, the supravital technique gave results indicating a similar origin in the

chief cells seen in these two conditions. Contrariwise, the scrapings of the two syphilitic juxta-articular nodes showed highly phagocytic cells which were capable of taking up the neutral red stain; these were regarded as either clasmatoocytes or highly stimulated monocytes. McEwen also found a few small oval cells similar to those seen in the nodules in rheumatic fever and rheumatoid arthritis but none of the characteristic large oval cells predominant in the latter lesions and no free spindle-shaped forms. Small numbers of oval and spindle-shaped elements were also found in the control material of normal subcutaneous tissue and these cells were more abundant in the connective tissue reaction around the tophus and sebaceous cysts. In the latter two conditions, however, the cells failed to show differentiation into the larger and occasional multinucleated forms seen in the nodules in rheumatic fever and rheumatoid arthritis. These observations led McEwen to the interesting conclusion that, although in rheumatic fever and rheumatoid arthritis there is a marked increase in the number and size of the young connective tissue cells, with their tendency to develop into the large and multinucleated forms, these "show little differentiation as far as the capacity to react to neutral red and Janus green is concerned." This property of the giant cells served to distinguish them from the similar clasmatoocyte of syphilis and the epithelioid cell of tuberculosis and, contrariwise, "it indicates that at least the proliferative phase of the tissue reaction is similar" in the nodules in rheumatic fever and rheumatoid arthritis.

Granting that this type of response is similar or even identical in the subcutaneous lesions in rheumatic fever and rheumatoid arthritis, it seems hazardous to go beyond this conclusion. That the data derived from the supravital method of studying the rheumatic nodule may be used *pari passu* to shed light on the derivation of the cellular component in the Aschoff body seems reasonable, though this may not be wholly without objection (16, 274). It seems doubtful that an etiologic alliance can be established on the basis of an identification of a single or more cells which probably represent various stages in the development of the primitive mesenchymal series ("different kinds of functional activities within a single strain of cell") (295). As the method is constituted at present, it appears to be more qualitative than quantitative, and exceptions will undoubtedly be encountered, depending

on the stage of development of the tissue and perhaps other factors as well. Thus, Pierce and Rosahn (296) found that in the early orchitis or scrotal chancre of experimental rabbit syphilis, the most conspicuous cell was the monocyte and that only later did clasmato-cytes appear in large numbers. Cunningham and Topkins (297) noted that "stimuli other than the tubercle bacillus can inaugurate production of epithelioid cells, even when defined in the terms of the more specific morphology permitted by the supravital technique." Most of the investigations have, thus far, been carried out in animals under experimental conditions, and it is not clear how far the conclusions may be applied to homo sapiens. McEwen's valuable studies which, it is evident, were carefully controlled, are of great interest as they were executed in conditions affecting the human being. There remain, however, other diseases which must be studied in a similar manner—to mention only one, the advanced lesions of granuloma annulare. In the last analysis, conclusions drawn on morphologic investigation, especially when based on the qualitative or quantitative identification of one or more cells, carry with them a definite hazard. This was recognized by McEwen whose data were only tentatively and presumptively applied. Confirmation of the results thus far published, using large numbers of cases and extending the investigations, would be of value, for as pointed out by McEwen, there is reason to believe that the data furnished by this method will aid in differentiating the syphilitic juxta-articular nodes from the clinically similar lesions in rheumatoid arthritis.

2. *Experimental reproduction of nodules:* It is conceded by critical investigators that, thus far, the numerous attempts to reproduce the Aschoff body in the heart, under experimental conditions, have been uniformly unsuccessful. This conclusion also seems to apply in the case of the rheumatic subcutaneous nodule, though much less work has been done on this phase of the subject. The major difficulty, as pointed out in this monograph time and again, is that there are no pathognomonic criteria for the recognition of the rheumatic nodule as observed in the subcutaneous tissue. Under such circumstances the most that can be expected is the reproduction of features resembling these lesions. Nevertheless, the investigations pursued up to this point have yielded interesting results that merit analysis.

In 1928 Clawson (298), injecting different strains of streptococci in varying amounts and at irregular intervals into the subcutaneous tissues of rabbits, produced what he called proliferative nodules in some cases. These were believed by him to be the analogues of the rheumatic nodules as observed in human beings. The conclusions reached by this investigator were not accepted by many pathologists (15, 290).

Using the *Streptococcus viridans* isolated from a case of subacute bacterial endocarditis, Moon and Stewart (1931) (299) asserted that they had reproduced, in the ears of rabbits, lesions regarded by them as the equivalent of the genuine rheumatic subcutaneous nodules. The conclusions arrived at by these observers are susceptible of the following criticisms: (1) the lesions in the ears of their rabbits merely roughly simulated the nodules in human beings, both clinically and pathologically; (2) they assumed that the subcutaneous tissues are capable of giving rise to changes specific of rheumatic fever under any circumstance. In addition, Aschoff (290) was unable to grant that these investigators had produced alterations which could be considered as rheumatic beyond cavil.

More recently Rinehart (278) claimed that he had observed nodules about the joints in animals subjected to a diet deficient in vitamine C and to a superimposed streptococcal infection. His illustrations are, however, not convincing and the results seem doubtful so far as their interpretation goes (274).

The investigations carried on by Massell, Mote and Jones (300) are of unusual interest in many respects. Basing their work on the principle that trauma is an important element in the origin of the rheumatic subcutaneous nodule, they attempted to reproduce these lesions by introducing the patient's blood into the subcutaneous and deeper tissues about one elbow following preliminary anaesthetization with novocaine. During the next 10 days frictional pressure was applied by having the patient rub the injected elbow on the bed-clothes a few times a day for several minutes or until the skin became warm. This constituted the fundamental procedure and it was by this means chiefly that subcutaneous nodules were formed about the second and third weeks of observation, these being regarded as clinically indistinguishable from those seen in rheumatic fever. This method was used

in 82 patients suffering from rheumatic fever or chorea in various stages of activity; 34 other subjects were used as controls. As only one elbow was injected in this procedure, the other one could be used as a second type of control (novocaine without, and later with, friction; friction alone; blood alone; and normal saline with friction). The following results were obtained. Of the 82 subjects with rheumatic fever or chorea, nodules were produced in 37 or 45 per cent of the sites treated by the basic procedure. Of the 34 patients free from rheumatic disease, only one developed a definite nodule and in this case there was questionable evidence of rheumatic heart disease. Moreover, in the rheumatic patients it was found that friction alone was not a sufficient stimulus in any case, that in some instances the injection of blood alone might cause formation of nodules but that novocaine, with or without friction, was inadequate to accomplish this result. However, the introduction of normal saline per se caused nodules to appear in several cases and in order to explain this discrepancy it was assumed that the injection had been accompanied by bleeding from rupture of vessels. It is, however, clear that this additional factor was considered as absent in the patients given novocaine. These interesting results were rendered even more remarkable by the ability of these observers to correlate the incidence and the size of the nodules with the degree of activity in the rheumatic process. In their next paper (301) these investigators, after a preliminary review of the literature on the minute anatomy of the rheumatic subcutaneous nodule, concerned themselves with the histologic alterations shown by the induced lesions. In general, they accepted the changes already outlined in this monograph, recognizing that the descriptions furnished in the literature were variable and sometimes conflicting; these variations they attributed to the different stages studied. Without going into great detail, it appears that some of the features ordinarily seen in the rheumatic subcutaneous nodule were found. That this establishes an identity between the two lesions in question seems doubtful for several reasons: (1) the typical structure of the rheumatic subcutaneous nodule was merely simulated; (2) the pathologic alterations in either lesion are not necessarily pathognomonic of any disease; (3) it has been recognized by Collins (214) that "a type of fibrinoid necrosis can occur as an effect of trauma", this appearing at sites of

intense edema in some cases. The unusual feature of the painstaking investigations pursued by Massell, Mote and Jones was that the control patients did not show these nodules following the use of the basic procedure, except in one isolated instance in which the diagnosis was uncertain as regards the possibility of rheumatic heart disease. Even though there is reasonable doubt that the typical rheumatic subcutaneous nodule was actually reproduced, these investigations are important, if only to emphasize the point regarding the variability of anatomical changes in lesions of different ages. Their interpretation of the results obtained by them would undoubtedly be aided by confirmation from other sources, using similar methods of control and recognizing the difficulties in their evaluation.

C. Juxta-articular nodes of syphilis

Until recent years (1918-20) the syphilitic juxta-articular nodule remained unrecognized as an entity. This curious fact may be attributed to: (a) its comparative rarity; (b) its occurrence, as a rule, in the tertiary stage of the disease, without being accompanied necessarily by other syphilitic manifestations, rendering diagnosis difficult in the pre-Wassermann-test era; (c) its unusual attributes, as contrasted with other syphilitic lesions; (d) its resemblances to the similar manifestations of rheumatoid arthritis and rheumatic fever, the analogies in the clinical picture being promoted by the occurrence of pains (joint pains?, periostitis?) and occasionally fever; (e) lack of co-operation between internists and dermatologists.

In 1884 Fowler (168) described the case of a man 35 years of age, who presented many persistent subcutaneous nodules, ranging in size from that of a pea to that of an olive. As the patient had not complained of articular pains, the relation of the lesions to rheumatism was denied; the term rheumatism was used in the broad sense of joint disease, and the nodules differed in many particulars from those characteristic of rheumatic fever. Discounting a previous history of a penile sore (10 years before), Fowler surmised that the condition was "probably not syphilitic." In the year prior to the appearance of this publication, Duckworth (169) recorded the occurrence of recalcitrant subcutaneous nodules in a woman 38 years of age and, on the basis of a history of "rheumatism" in the immediate family, considered

the manifestation as of rheumatic nature. No mention was made of other evidences of syphilis, but it was noted that the lesions softened and diminished in size during the administration of iodides over a period of 3 months. In the same year (1883) S. Mackenzie (170) recorded a case in which stationary nodules were associated with the occurrence of papular syphilides on the forearms and right scapula and a serpiginous eruption on the feet and nates. Despite a previous history of a vulval sore appearing some three years before, succeeded in turn by persistent tonsillitis and the subsequent occurrence of 2 miscarriages and 2 stillbirths, Mackenzie did not attribute the nodules to syphilis, but reported the case only because of the absence of rheumatism (articular pain). Following Mackenzie's publication, Duckworth (171) was now able to elicit from his patient a history of a previous generalized rash "like measles" accompanied by sore throat, both disappearing following the use of pills said to contain sarsaparilla. In 1895 Fitcher (77) recorded a remarkable example of juxta-articular nodes in a colored patient 49 years of age. This case is of such wide interest as to merit a summary of its salient features. Ten years before admission, the patient acquired gonorrhea. There was no history of concomitant syphilitic manifestations. Two years later a small lump appeared under the skin above the right knee and 4 months later a similar lesion was found on the opposite knee, then over both elbows. The nodules grew steadily until one year before hospitalization, when they remained stationary in size. Three years before admission there was severe pain in the left foot which was markedly swollen and tender, confining the patient to bed for 2 weeks. The chief complaints on admission were dyspnea and pain in the left chest, with radiation to the left shoulder. There were two large subcutaneous, freely movable, lobulated tumors on the left elbow just below the tip of the olecranon. The lesion measured 3 cms. by 4 cms. in diameter. There were similar, but larger, nodules about the knees. The parotid glands were symmetrically enlarged. There were clinical signs of an aortic aneurysm, the evidence becoming more definite as time went on. Post-mortem examination revealed aneurysms of the ascending, transverse, and thoracic portions of the aorta. Microscopic study showed that the aorta was the seat of an "extensive nodular endarteritis." The attributes of the subcutaneous nodules and the postmortem findings

clearly indicated that this was an instance of syphilitic juxta-articular nodes, apparently the first case reported in the American literature and one of the few examples accompanied by a necropsy report. Even as late as 1926 there were many excellent observers, for example Jeanselme (302), who did not recognize the distinctions between the syphilitic juxta-articular node and the similar lesion in rheumatoid arthritis (318, 319). As will be shown later, on the basis of the large casuistic literature which has grown steadily since 1920, the conception of the syphilitic node as an entity rests on three solid foundations; (1) association with other manifestations of syphilis; (2) the almost invariable presence of a positive Wassermann reaction; (3) the striking response to anti-syphilitic treatment. In many cases all three evidences may be found in the same patient. These, rather than the results of pathologic examinations, have been the criteria for the recognition of the lesions.

(a) *Clinical features*: The attributes exhibited by syphilitic nodules are usually distinctive without in any way being pathognomonic. They are more apt to be confounded with the large persistent tumors seen in rheumatoid arthritis, rather than with the smaller transient lesions of rheumatic origin.

The localization of the syphilitic tumors about the joints has given them the descriptive title of juxta-articular nodes; in this respect, however, they do not differ strikingly from the lesions in rheumatoid arthritis. The extensor aspects of the limbs are especially favored. While they are usually situated near an articulation, they are generally found a short distance from it, in areas where only a thin layer of tissue is interposed between skin and bone (172). As in the case of rheumatoid arthritis, observers have stressed the element of trauma in the origin of these nodules. Sites of predilection are the regions about the elbow (olecranon), knee (tibial tuberosity), and hip (greater trochanter?), less commonly the ankle, shoulder, sacrum etc. Abar-ticular locations are, however, also affected in occasional instances; for example, the forehead, forearms, buttocks, palms (personal observation) and elsewhere. Hopkins (165) stated that in his experience the syphilitic nodes were usually limited to the regions of the elbows, whereas in those cases accompanied by arthritic complaints, generalization of the lesions was more apt to occur. In all three examples

under my observation, the neighborhood of the olecranon process was affected; in two, this was the only area involved and in the third instance, accompanied by articular manifestations, the palm was also implicated. It appears, then, that the regions of the elbows are rarely spared. Usually the nodes are bilaterally symmetrical from inception; occasionally there is a single lesion, but fresh nodules may appear subsequently on the contralateral side, sometimes even after 20 years or more have elapsed. In some instances asymmetry may be caused by disappearance of lesions from one limb, as attested by the presence of scars from previous ulceration through skin.

In contrast to rheumatic nodules, the syphilitic variety has an average size of a walnut, but the magnitude of a hen's egg is commonly attained. However, in occasional instances the syphilitic lesions may be as small as a pea. The nodules are oval, spherical or hemispherical; they may be irregularly lobulated from coalescence of several smaller lesions. Fresh nodes are apt to be soft in consistency, but they tend to become firm with age. This acquired attribute of firmness, stressed by many observers (173) (personal observation), is by no means pathognomonic of the syphilitic juxta-articular node.

In the majority of cases the overlying skin is unaltered, but there are many instances where the integument has been thinned out, assuming thereby various shades of discoloration. Adherence to skin is, therefore, not uncommon. In occasional patients scars may appear as a result of ulceration through the integument, a phenomenon foreign to the rheumatic nodule.

The syphilitic lesion is characterized by slow development, requiring months and even years to attain maximum size. Hoffmann (172) stressed the point that several years may elapse before it becomes as large as a walnut. In contrast to the rheumatic nodule, the syphilitic node may persist over 35 years (174). Spontaneous resolution, in the absence of ulceration of the administration of anti-syphilitic therapy, is uncommon.

Corresponding to the age incidence of the disease, the syphilitic lesions are generally encountered in persons between the ages of 30 and 50, whereas the rheumatic subcutaneous nodules are principally found in children and young adults. The juxta-articular nodes usually appear in the tertiary stage of syphilis, commonly in association

with other cutaneous lesions, such as tubero-serpiginous syphilides (personal observation) (170, 173, 218, 222, 303-305), gummas (305, 306), and, occasionally also, with such visceral manifestations as cerebral syphilis (307, 304) and aortic disease in various phases of development (304, 305, 77). These nodes may occur, though less commonly, in the secondary stage of the affection, accompanying a papular syphilide (305), condylomata lata (172), and leucoderma of the neck (305). No instances have been recorded as appearing in the primary stage or during the course of proven congenital syphilis. According to Rossow (305) whose statistics are based on an interesting group of 20 cases collected from the Russian literature, these lesions appear in from $2\frac{1}{2}$ months to 23 years after the initial acquisition of the infection. The large casuistic literature is one of the solid foundations on which the etiology of these nodules has been based, notwithstanding the almost constant failure to demonstrate the spirocheta pallida. Practically all the cases recorded in the literature have been featured by a positive Wassermann reaction at the time of examination, with but rare exceptions (307, 308). In Lane's patient (309) there was a previous history of a positive reaction for which mercurial treatment had been given for only a short time. Investigations to discover the spirochete have been practically always fruitless. In one isolated case, recently reported by Jessner and Rossiansky (310), a positive result was obtained in a second generation of rabbits, using the method of multiple inoculation (Dr. Fried). Even in the juxta-articular nodes caused by framboesia, where the spirochaeta pertenuens has been demonstrated in rare instances, this examination has nearly always been negative.

(b) *Therapeutic responses:* In the several cases under my observation, anti-syphilitic treatment caused an immediate and striking diminution in the size of lesions. In one instance this was noteworthy as the histologic examination revealed that the major part of the removed specimen was composed of what was interpreted as fibrous tissue. These results are not isolated, for similar success has attended the use of various anti-syphilitic remedies (neosalvarsan, bismuth, iodides, merurials) by a host of observers (169, 304-306, 311-313). The older literature contains the reports of many cases where iodides were given for therapeutic purposes. It is evident that the involution

of such lesions during the administration of this drug may have no great significance, if their average course is one of short duration. Thus, Meynet (9) justifiably refused to credit this medication with the disappearance of the rheumatic subcutaneous nodules seen in his case, as he suspected the possibility of spontaneous resorption. On the other hand, when recalcitrant lesions, like the juxta-articular nodes, are favorably influenced by iodides, as in Duckworth's case (169), the observation assumes great significance as regards a possible syphilitic origin. Contrariwise, there are many examples of painful subcutaneous lesions appearing on the lower limbs of patients suffering from syphilis, where the eruption was subsequently proven to be caused by the intake of iodides. Each case must therefore be individualized.

It seems pertinent to inquire as to how far the "therapeutic test" may be considered a reliable criterion for the diagnosis of the syphilitic juxta-articular nodes. The non-specific effects exerted by the arsenicals and other drugs are well known and occasionally this may be an important source of confusion. Yet in observing even a limited number of examples of these lesions and in comparing the results attained by others, one cannot but be struck by their uniformly rapid response to therapy, despite previous recalcitrance. Although relatively little is known about the effects of the various drugs mentioned on the clinical course of the nodules in rheumatoid arthritis, it seems justifiable to formulate the rule that, if in a doubtful case of this sort (and there are a few), there is no appreciable response to anti-syphilitic remedies in a short time, perhaps measured in weeks, the cause of these nodules must be sought elsewhere. This rule applies with particular force to lesions known to have exhibited the attribute of persistence. The question concerning the arthritic phenomena in syphilis, in its relation to the problem at hand, will be best considered after the pathologic data are presented.

(c) *Pathology of the syphilitic juxta-articular nodes*: Occasional observers (173) have regarded the minute anatomical alterations in these lesions as comparable to those in the gumma. The consensus of pathologic opinion, however, is that the changes noted are not pathognomonic of orthodox syphilis. In most cases where the pathologic diagnosis was that of "fibrosing" (305) or "organizing" gumma (173,

309), it is evident that the report was considerably influenced by knowledge of the clinical features. An exception was recorded by Truffi (311) in whose patient the principal microscopic changes in a nodule recalled those of fibroma; however, the relative wealth in small blood-vessels showing intimal closure and larger ones with thickening of the middle coat, notably marked in the veins, led Truffi to try anti-syphilitic treatment, following which there was striking involution of the remaining lesions. Nevertheless, most observers hold with Jessner (174), Hopkins (165), Kumer and Lang (222) and others who are unwilling to interpret the anatomical alterations as specific of syphilis in its accepted sense. It is interesting that Jeanselme (302, 320, 321), in describing the pathologic changes in these lesions, noted the division into 3 areas corresponding in a degree to those mentioned in the section under rheumatoid arthritis. Kumer and Lang (222) who made comparative observations on tropical and non-tropical juxta-articular nodes, stated that the minute anatomy was indistinguishable in the various examples studied by them. Welti (218) found that the argyrophile fibers were preserved in the central necrobiotic zone, as in rheumatoid arthritis, an observation substantiated by Kumer and Lang (222) and Wolf (312). The necrobiotic areas and the vascular lesions in the smaller arterioles are comparable to those found in rheumatoid arthritis and, to a lesser extent, in rheumatic fever. Endophlebitic changes are relatively uncommon, but were a conspicuous feature in Truffi's case. Various degenerative changes, such as calcification, xanthomatous transformation etc., have been noted in a fair percentage of nodes. According to Kumer and Lang, the advent of healing is indicated by (1) a decrease in the cellularity of the lesion; (2) the disappearance of basophilism in the cytoplasm; (3) appearance of fresh collagen bundles at the edge of the necrobiotic zone; and (4) the absence of argyrophile fibers in the centrally located scar tissue.

McEwen's studies are of special interest. In the examination of 2 syphilitic juxta-articular nodes by routine methods of staining, he found that the vascular alterations were less pronounced than in the case of nodules in rheumatoid arthritis and rheumatic fever and that a few small vessels showed moderate thickenings but not the characteristic features of syphilis. His supravital studies which have been mentioned already, may provide a means of differentiating the syph-

ilitic tumors from the other varieties of nodules likely to be confused with them. The predominant cell found by McEwen in the former lesions was a highly phagocytic clasmatoocyte, confirming the observations made by Morgan (314) in the experimental syphilis in rabbits. The entire subject needs more intensive study, and if the findings recorded by McEwen are confirmed, a method of investigation will be available affording scientific means of solving what may occasionally be a vexing problem.

(d) *Arthritic phenomena, subcutaneous nodules, and syphilis:* There are cases where it is exceedingly difficult, if not impossible, to distinguish between syphilitic juxta-articular nodes accompanied by "arthro-lues" and ordinary rheumatoid arthritis accompanied by its subcutaneous lesions and a coincidental latent syphilitic infection. The controversy between Hopkins (165) and Dawson (213) regarding the status of such cases shows that even excellent men may interpret a clinical picture in various ways; as in many controversies of this sort, there is a kernel of truth on each side. Hopkins cited two interesting cases that bear on this discussion. In one instance a woman 41 years old had suffered from "chronic infectious polyarthritis" for a long time. Under observation, juxta-articular nodes appeared. The Wassermann reaction was negative; yet, under silver arsphenamine the lesions diminished to one-half their original size (non-specific effect?). In the other patient, a man aged 40, who was also known to be afflicted with chronic infectious polyarthritis, there was a history of a chancre and the Wassermann test was positive. There was a generalized distribution of subcutaneous nodules which were, however, uninfluenced by the administration of arsphenamine and iodides. Hopkins regarded the subcutaneous lesions as belonging in the category of rheumatoid arthritis, an opinion which seems to be justified. However, in stressing the unusually high incidence of syphilis in his group of cases, as contrasted with Dawson and Boots, Hopkins apparently over-emphasized the importance of this factor and this may be attributed to the peculiar nature of his material. On the other hand, the justice of Dawson's criticisms of such instances may be freely granted, but, here again, it appears that this observer ventured too far in denying the clinical and pathologic similarities (by the ordinary methods of examination) between the nodules in syphilis and rheumatoid arthritis.

The points cited by Dawson in support of his contentions regarding the "rheumatic nodule" have been shown to be inadequate. In addition to the cases mentioned in the text, there are a few others which may be discussed in this connection. Thus, Stern's case (303), featured by the occurrence of a juxta-articular nodule about one elbow, tubero-serpiginous syphilide and minute anatomical changes strongly resembling those in rheumatoid arthritis, had no previous evidence of articular involvement in any form. Welti's patient (218) had "chronic rheumatism" of the left knee for many years; this manifested itself about the time the nodules appeared and, when observed, there was present, also, a tubero-serpiginous syphilide. In Jordan's case there was a history of what seems to be typical rheumatoid arthritis; in addition, the patient had a positive Wassermann, gumma on the left tibia, and juxta-articular nodules that responded rapidly to bismuth and iodides. It is possible that this was a case of rheumatoid arthritis complicated by syphilitic nodules. Finally, there came under my observation a woman who had complained of generalized arthritic pains for some time. These were not followed by any visible changes in the joints but some time later subcutaneous nodules appeared about the elbows and in one palm; the Wassermann reaction was positive and the nodes disappeared under neoarsphenamine and bismuth. Such cases seem to substantiate the belief in the syphilitic origin of the juxta-articular nodules, irrespective of whether they are associated with joint pains. The nature of the joint manifestations is often difficult to classify and each case will have to be individualized.

D. Fibrositis and panniculitis

The host of conditions assembled under these designations are at present attended by a haze of obscurities regarding concept of disease, pathologic changes, and etiologic factors concerned. They have been considered as of "rheumatic" origin but it appears that the infiltrations described in these conditions have no relation to the rheumatic subcutaneous nodules.

The clearest exposition of the concept embraced by these terms is that given by Stockman (175) who defined fibrositis as a condition in which there is chronic inflammation of the white fibrous tissue of fasciae, aponeuroses, sheaths of muscles and nerves, ligaments, tendons,

periosteum and subcutaneous tissue, with the production of infiltrations causing pain, aching and other "rheumatic" symptoms. The term panniculitis was reserved for a similar process restricted to the subcutaneous tissue (chronic subcutaneous fibrosis). Stockman described various forms of circumscribed and diffuse indurations, these being characterized by the occurrence of pain, particularly when pressure was applied to these lesions. The painful state was attributed to perineuritis of the fine sensory nerves which are alleged to be implicated in the process. Microscopic examination of a nodule reveals inflammatory hyperplasia of the connective tissue, accompanied by formation of many newly-formed capillaries, with thickening of the parietes of larger vessels. The earliest change is the exudation of a sero-fibrinous material; the later stages are characterized by organization and fibrosis. The lesions are especially encountered in the lumbar aponeurosis, fascia lata and other parts of the body. Among the various causes listed by Stockman, rheumatic fever is given prominence on the basis of indefinite evidence and broad interpretation.

Bain described panniculitis as occurring in small circumscribed patches, one or two inches in diameter, the principal sites of predilection being the supramammary regions in women, upper intercostal areas, abdomen, and fascia lata. Bain stressed the occurrence of hyperesthetic skin, rather than the presence of palpable indurations. On the other hand, Jausen (177) stated that the nodules of so-called panniculitis are, in reality, common fat collections appearing in persons who were previously subject to more or less obesity and had since lost weight; that the condition is practically restricted to women in the menopause; that the variable element of pain is attributable to nervousness; and that, when painless, the lesions are not generally considered as abnormal. In a wide variety of disorders seen in an orthopedic clinic Sutro (30) observed similar indurations which on microscopic examination proved to be circumscribed collections of fat. It is apparent, therefore, that there is marked disagreement regarding the essential pathologic alterations, some stressing the primary part played by white fibrous connective tissue, others attributing the condition to a peculiar, but fairly common, deposition of fat. The element of pain, emphasized by many observers, appears to be a frequent though variable factor.

Included under the designations of panniculitis and fibrositis are numerous other affections, ranging from the obscure "muscular rheumatism" (recently termed myogelosis by some authors) to dermatomyositis. It is interesting that the submiliary nodule (Coates and Thomas) was recently placed by Coates (178) under the category of fibrositis, in an attempt to relate acute and chronic "rheumatic infection". In a previous section evidence was presented indicating that these lesions could not be considered as an undoubted manifestation of rheumatic fever in its restricted sense (29), and that there was reason to ally these nodules with the non-specific adipose deposits described by many observers. Analysis of data recorded in the literature reveals that numerous other conditions have been grouped in this general category on the basis alone of involvement of subcutaneous fat; a discussion of these diseases will be found in other publications (28, 316). Rolleston's suggestion (179) that the syndrome of panniculitis may be related to rheumatoid arthritis is interesting but unproven. In any event, the feature of pain cannot be made the common denominator of conditions included under the heading of rheumatic fever.

E. Gouty tophi in adults

In the United States gout appears to be a relatively uncommon disease. However, there are observers (180) who claim that the incidence of atypical variants is remarkably high. That the affection may run its course in the absence of characteristic articular paroxysms is generally acknowledged, but it seems that in many instances the criteria for the recognition of the disease are too broad to meet with universal approval. As a rule, the diagnosis is based on the following features:

(a) The clinical picture or the history of a typical articular attack, the attributes of which need no mention. However, true gouty deformities of joints may sometimes appear insidiously without being accompanied by pain, as in one case under my observation.

(b) The uric acid content of blood exceeds 4 mgs. per 100 c.c. with associated diminished urinary concentration of that substance in the intervals between acute attacks of the disease. An increase in blood uric acid bears particular significance when the patient has been main-

tained on a purin-free diet for at least 3 days. To be sure, it is essential to rule out other conditions in which there is either increased destruction of nuclear material (leukemia, pneumonia) or retention from impairment of the concentrating power of the kidneys (azotemic phase). However, under certain ill-understood circumstances, it would seem that affections producing more or less sustained liberation of uric acid or its derivatives may precipitate an attack of gout in patients showing "constitutional predisposition" to the latter condition.

(c) The appearance of tophi at sites of predilection is most important for diagnosis. Thannhauser and Weinschenk (181) stated that they had not observed the occurrence of definite uratic deposits in the absence of an increase in the blood uric acid content. These observers were, however, cognizant of the point that transient diminution of uric acid in the blood may occur in patients on a purin-free diet or under the influence of atophan. There appear to be occasional exceptions to the view stated by Thannhauser and Weinschenk; for example, there came under my observation an unusual instance of gout, characterized at first by the development of a single subcutaneous nodule over a knuckle, the blood uric acid value remaining normal for 3 months after the deposition of uratic material. Because of the absence of a history of gouty paroxysms, the normal blood uric acid values on several occasions, and a roentgenogram showing no significant abnormalities, the condition was not recognized. One year later, with the interim appearance of generalized tophi, the uric acid content of the blood now ranged from between 6 and 12 mgs.; examination of aspirated material disclosed typical needles of sodium urate and the murexide test was positive. In still another case of gout under my observation, the blood uric acid remained within normal levels despite a typical articular paroxysm.

From the foregoing discussion, it will be seen that the only pathognomonic feature of the disease is the presence of visible tophi containing sodium urate crystals and giving a positive murexide test. According to Grafe (182), the finding of urate deposits in the internal organs constitutes a valuable confirmatory sign of gout, though it is not pathognomonic of the disease; this view is disputed by other observers. The roentgenographic evidence of subchondral defects may be

noted in forms of arthropathy other than gout. The several metabolic tests proposed by various men have not met with general acceptance, as they present difficulties both in their execution and in their interpretation (183). The delicate friction sound heard on movement of the knees and other joints was regarded by Goldscheider as a specific sign of gout, but this view has not been accorded universal approval.

In the older literature the clinical picture of gout was not uncommonly confounded with rheumatic fever (133), owing to the general use of the vague term "rheumatic gout". The designation "calcium gout", originally introduced by Minkowski to express clinical resemblances between foci of circumscribed calcinosis and tophi, was later broadened into a concept in which it was conceived that the syndrome depended on an analogous metabolic background. As the latter view lacks the confirmation of chemical studies and as the genuine gouty deposits may become secondarily infiltrated with lime, the term "calcium gout" requires clarification.

The physical attributes of the tophus differ strikingly from those seen in the typical rheumatic subcutaneous nodule, although both show similar predilection for certain areas of the body. It was previously noted that the rheumatic type rarely affects the ears (11, 55). Bourcy (90) recorded a characteristic example of rheumatic fever in which subcutaneous nodules were found about the articular ligaments of the fingers, apparently "simulating the gouty variety perfectly". However, the resemblances appear to end at this point.

1. *Clinical features of the tophus*: Tophi arise in a variable manner. Certain minor deviations in morphology are determined, to some extent, by the factor of location. Of 150 cases of gout, Duckworth (134) found them in 49 patients. Minkowski (183) estimated their incidence as from 20 per cent to 50 per cent.

Visible gouty deposits occur uncommonly during the initial attack of the disease, but are generally found only after a series of recrudescences. Occasionally they appear insidiously and independently of painful paroxysms; they may antedate onset of joint symptoms by many years. Cases showing visible or palpable tophi, in the absence of an articular attack throughout the course, are termed "atypical gout", but it seems necessary to require the demonstration of sodium

urate in such lesions, as clinical impression alone may not always be precise.

The affinity of urates for cartilaginous parts of points seems well established. The periarticular tissues are also sites of election; thus, the bursae, tendon sheaths, ligaments, and subcutaneous tissues in these regions are commonly affected, giving rise to visible tophi. Gouty deposits seem to favor the joints of the upper extremities, whereas the paroxysms appear to strike the joints of the lower limbs with particular violence, but this rule admits of numerous exceptions. In addition to predilection for joints, abarticular parts of the body are not infrequently affected, chief among which may be mentioned the ears; less commonly, the tips of the fingers, eyelids, nasal cartilages, cornea, penis and scrotum are involved. Excepting the eyelids and tips of the fingers, the tophus has its origin in the subcutaneous tissue, with secondary implication of skin. There seems to be a tendency to affect "mesenchymal" structures, but there are exceptions; for example, the supporting tissue of the heart appears to be spared. Numerous other diseases may also involve "mesenchymal tissue", and, while the use of such concepts affords a convenient way of regarding an entire disease, the designation must not be allowed to degenerate into a shibboleth.

At inception the deposit is soft and is usually accompanied by signs of inflammation, such as pain and a dusky pink or reddish color of the overlying integument. The element of pain is more likely to be encountered in tophi arising in the neighborhood of joints, whereas abarticular deposits are commonly asymptomatic. Not infrequently the nodule develops insidiously, without any accompanying reaction on the part of the enveloping skin. When the lesion is large, the fluidity of its contents may give rise to the clinical signs of fluctuation. The subjacent deposit can often be seen glistening through the skin as whitish or yellowish areas, especially in older lesions, an effect made more evident by rendering the affected parts taut. Gradually the nodule becomes firm, at times creating the impression of an embedded foreign body. An increase in size of tophi may occur insidiously, from fresh deposits being laid down during a paroxysm of the disease, or from coalescence of adjacent lesions. Such tophi often assume unusual magnitudes, form knobby protrusions, frequently soften,

reveal points of fluctuation, and may break down with the extrusion of mushy, crumbly, chalky-like masses. When secondary infection sets in, persistent "gouty ulcers" form, healing slowly and frequently requiring surgical eradication for cure. The presence of small cicatrices at sites of predilection may sometimes constitute a lead toward diagnosis of gout, but similar scars are also observed in calcinosis and other conditions. On experimental grounds, Freudweiler (184) showed that urate deposits may resorb spontaneously through the process of phagocytosis and probably also, from chemical and physical means. Umber observed this phenomenon clinically, stating that even large fresh tophi may undergo complete involution after an attack of gout. However, the rapidity of disappearance in no wise equals that which may be seen in the rheumatic subcutaneous nodule. On the other hand, Minkowski (183) stated that complete absorption occurs as a result of organization or from secondary rupture and emptying of the contents of lesions.

Certain variations in physical attributes appear to be correlated with different sites of predilection. These will be considered seriatim.

a. *Ears*: Gouty deposits appear to favor the upper parts of the ears, the usual site being the helix; less commonly, the antihelix; and rarely, other areas. The lesions may be single or multiple, and occasionally coalesce to form knobby protrusions. Usually there is no associated pain and the inflammatory signs are minimal or absent. Exceptionally, symptoms may occur, similar in principle to those of a gouty paroxysm (185). The lesion arises in the subcutaneous tissue, forming a movable mass that is characteristically independent of subjacent cartilage, a point corroborated by roentgenologic study. The nodules may break down spontaneously or as a result of varied types of trauma. It is noteworthy that gouty deposits in the ears rarely become secondarily infected, and they may be punctured with impunity for diagnostic purposes. Not infrequently the nodules antedate onset of arthritis by years and, under such circumstances, may constitute the "revealing sign" of a "gouty disposition". It is necessary at all times to demonstrate the crystals of sodium urate and a positive murexide test, as the lesion may be confounded with cysts of various types, milia, and other similar structures. The painful nodules (chondrodermatitis nodularis chronica helices) (186, 187) on the

upper pole of the pinna, on or near the free border of the helix, bear only superficial resemblances to the tophus; this is also true of the similar lesions encountered on the antihelix, as described by Klauder (188). The so-called chondrogenous tophus found chiefly on the antihelix (189, 190) is distinguished from true gouty deposits in this situation by the absence of sodium urate and by primary involvement of cartilage; the data presented to indicate a gouty origin for these lesions are not conclusive.

(b) *Bursae*: Involvement of bursae is a frequent occurrence in gout. The most common site is probably the region of the olecranon process; less commonly, the parts about the patellae, malleoli, and the lower end of the Achilles tendon. Goldscheider (180) stressed the importance of bursae as sources of urate deposits, particularly emphasizing those structures situated in the region of the sacroiliac joints; it is likely that this sign, though a valuable one, was given undue significance (83, 183). Indeed, Gudzent (83), performing biopsies on such lesions, found that they were composed of connective tissue alone, without deposition of urates. Involvement of structures about the sacroiliac joints may be found in conditions other than gout, as in one instance of rheumatoid arthritis under my observation.

Deposition of lime is frequently superimposed, interfering with the demonstration of sodium urate. Thus, there was encountered a case in which a specimen of material obtained from an olecranon bursa showed only calcium, whereas a second examination revealed typical crystals of sodium urate and a positive murexide test. However, calcific deposits in bursae do not *per se* warrant the diagnosis of gout, as similar findings may be encountered in connection with other conditions. Goldscheider's belief that urates may disappear in the course of time appears to be supported by experimental evidence (184), but the clinical application of this observation seems to possess restricted value. If, after careful persistent search, sodium urate cannot be demonstrated in a nodule, it becomes necessary to seek elsewhere for evidence of gout.

(c) *Arthritic tophi*: In this type the uratic deposits seem to arise in relation to structures immediately surrounding joints. Frequently the swellings produce large knobby projections, particularly on the fingers. Such lesions may lead to the formation of bizarre deformities

of the affected parts. True or pseudo-fluctuation is commonly associated and scars are often seen consequent upon the breaking down and emptying of the contents of nodules.

Garrod (101) described a syndrome characterized by the occurrence of pads on the dorsal aspects of the proximal interphalangeal joints in association with Dupuytren contracture. Although the latter condition was regarded by Duckworth as the "appanage of gout," it appears that a relation between them has not been established. A more complete account of these interesting lesions is given in Weber's recent paper (315).

The ordinary Heberden node may at times be simulated by true gouty deposits containing sodium urate.

The tiny scars observed on the fingers of patients afflicted with gout possess only suggestive value for diagnosis, as identical cicatrices may be found in circumscribed calcinosis and other conditions.

2. *Pathologic features:* In several recent communications Weil and his co-workers (219, 317), studying the minute anatomy of tophi, stated that "one cannot but be struck by the analogies which exist between their structure and that of the rheumatic nodules." They noted the occurrence of the same degenerative and exudative phenomena early in the course, succeeded later by connective tissue proliferation and granuloma formation around the central zone of necrobiosis. They regarded the "fibrinoid necrosis" as an essential feature, which was followed by "encystment", the name given to the proliferation of cells of the connective tissue series at the periphery. The one distinguishing point in this process was the deposition of sodium urate crystals which they found early in the course. The uratic deposit was considered by them as formed secondarily to the initial inflammatory process, a conclusion which brings to mind Ebstein's original view. On the other hand, Freudweiler (184) showed, on the basis of extensive experimentation, that sodium urate can be toxic to tissue, causing damage which may eventually lead to necrotic changes. As a consequence, inflammatory cells are called forth, these being succeeded or replaced by fibroblasts in later stages. Foreign-body giant cells may also be encountered about fragments of tissue. Occasionally there is a secondary deposition of cholesterol (191, 317) or of calcium (317). The surrounding vessels may present changes designated

as arteritis, endarteritis, and periarteritis. It will be seen, then, that aside from the presence of sodium urate crystals, there is little that permits recognition of this lesion in ordinary pathologic examinations. As there is evidence that this substance may be spontaneously absorbed, it would not be surprising to find lesions, especially those existent for many years, where the demonstration of these crystals is difficult. In their absence, however, the diagnosis of gout can only be suspected, and it may be necessary to examine other lesions. The type of reaction, if one adheres to Freudweiler's views, seems similar to that observed in many conditions involving a foreign body concurrently toxic to tissue, for example, lycopodium granuloma (192).

McEwen's studies (265) with supravital methods on one tophus revealed small numbers of oval and spindle-shaped elements in the scrapings, but the cells failed to show differentiation into the larger and occasional multinucleated forms seen in the nodules in rheumatic fever and rheumatoid arthritis. It would be interesting to extend these observations, notably to lesions of a relatively young age.

F. Subcutaneous nodules in periarteritis nodosa

Varieties of nodular infiltrations have been recorded in association with periarteritis nodosa, but it is difficult to determine whether all deserve to be placed in a single category. For the purposes of this report, discussion will be restricted to typical examples of the subcutaneous nodules seen in this condition. Before considering this subject, it seems desirable to review certain general features illustrating the present obscure status of the disease.

Although the diagnosis of periarteritis nodosa may be suspected clinically (212) and even occasionally substantiated postmortem, the concept rests for the most part on an anatomical basis. It appears that the pathologic changes represent a peculiar reaction of blood vessels of a certain type and calibre to a variety of undetermined toxic and infectious causes, rather than an autonomous disease (193). That dissension still exists regarding the microscopic criteria for diagnosis may be gathered from Plaut's (194) cogent statement: "I should not be astonished if twenty years from now many lesions that were previously listed under periarteritis nodosa are reclassified." Excluded from this category are localized forms of degenerative arterial

changes observed in many infectious diseases (195, 196) and mycotic aneurysms. More difficult to classify are certain types of generalized arteritis and necrotizing arteriolitis. Though the future may unearth transitional cases, it seems wise, at present, to adopt strict morphologic criteria for the microscopic diagnosis of periarteritis nodosa. As minimum evidence, it appears necessary to demonstrate simultaneous occurrence of alterative (degenerative) and inflammatory changes in localized stretches of middle-sized and, perhaps, slightly smaller arteries. The former type of alteration is represented by necrosis of the media, leading to formation of characteristic rings or sectors of necrobiotic tissue, with frequently associated fragmentation of the internal elastica and deposition of fibrinoid material; in the opinion of Schultze (197) and others, this is the one fundamental obligatory feature. Less important, perhaps, but equally necessary for diagnosis, according to other pathologists, is the presence of inflammatory cells in the adventitia and, to a lesser extent, in the media, producing periarterial infiltrations. In the early stages the inflammatory response is acute, with the presence of numerous polymorphonuclear leucocytes; the occurrence of eosinophiles in large numbers is a characteristic, but not a constant, feature of the cellular reaction. In later stages there may be subintimal proliferation leading to formation of a thickened intima, with replacement of the acute cellular infiltration by fibroblastic cells and granulation tissue, evidently an attempt at repair. The closure of vessels by intimal thickening or from secondary thrombosis, with or without evidence of recanalization, and the formation of aneurysmal dilatations owing to weakening of the media are important secondary phenomena that may give rise to recognizable clinical features, but their occurrence is facultative. As the presence of media necrosis appears to be fundamental, it follows that the arteries showing characteristic changes must be of such calibre as to contain appreciable amounts of muscle tissue; otherwise, this outstanding feature will be lacking. This point is of considerable importance, for it seems probable that structural alterations strictly comparable to those of periarteritis nodosa will be recognized principally in vessels of the size seen in subcutaneous tissue and, possibly also, in the lower limit of the cutis bordering on the fatty layer; the vasculature of the remaining portion of skin being relatively devoid of

media, the changes found are likely to be of a nonspecific type, simulated by other conditions. The characteristic band of media necrosis may be imitated by intense edema of the parietes, as observed in erythema nodosum and other affections, but such alterations do not warrant inclusion in the group of periarteritis nodosa, at least not on such evidence alone.

In the internal organs the middle-size coronary, renal, intestinal, and, more rarely, the pulmonary, pancreatic, and cerebral vessels etc. are affected. The pathologic changes of periarteritis nodosa appear to be restricted to arteries, the veins being spared or, rarely, involved by secondary extension of periarterial inflammation. In the average case the vascular lesions are scattered throughout many organs of the body, with particular predilection for the kidneys and heart, and, to a lesser extent, in the gastro-intestinal tract, gall bladder, pancreas, liver, brain, testicles and other structures. Not only are the lesions widespread, but within any particular organ affected there are many vessels showing the characteristic changes. Until the evidence becomes more definite, it seems wise to exclude examples where arterial lesions are found with difficulty in isolated organs postmortem, and only after prolonged search.

In the average instance of periarteritis nodosa there is gross evidence of arterial disease, leading to formation of fairly large infarcts; the course of the affected vessels may stand out in bold relief owing to the presence of nodules (aneurysms) or of focal thickenings of the parietes. Cases have been described where there was no macroscopic evidence of vascular disease or its secondary consequences; yet, study of the minute anatomy disclosed typical lesions. However, these instances are relatively uncommon, and a review of such material often reveals gross changes that have been admittedly overlooked (Lamb's case 1) (210). Considering the size of the affected vessels and the nature of the anatomical alterations, the presence of gross change is to be expected in practically all cases.

The prognosis of the average instance of the condition is poor or guarded and death occurs in most patients in a relatively short time, generally measured in months or years. Alleged instances of recovery have been recorded, but the precise status of many of them has not been established beyond a doubt. Where healing or healed lesions

have been described, the patient generally died from the secondary effects of the process (thrombosis with infarction, aneurysmal formation with rupture etc. etc.). Until general agreement is reached regarding the precise limits in the conception of this affection, it will be difficult to gather accurate data bearing on the prognosis.

Finally, there appeared recently a paper recording 4 cases of periarteritis nodosa associated with rheumatic heart disease, where microscopic examinations revealed typical Aschoff bodies in the heart (211); it seems that rheumatic fever may produce vascular lesions of the type seen in periarteritis nodosa. It has already been noted that there are observers (268) who believe that the vascular changes reported by Von Glahn and Pappenheimer in rheumatic fever (260) actually belong in the category of periarteritis nodosa. In discussing the fundamental resemblances between these conditions, Klinge and Vaubel (268) noted that in rheumatic fever these alterations in the blood vessels must be searched for assiduously; on the other hand, they emphasized the point that in periarteritis nodosa the vascular changes are found, without disturbances in the general mesenchymal tissue as in rheumatic fever, but the cases examined thus far are too few to permit generalization on the basis of this differential feature. The example reported by Klotz (205) merits notice as an instance in which the patient gave a previous history of rheumatic fever and in which postmortem examination revealed what appeared to be old rheumatic heart disease, with no evidence of recent activity. How common this association of conditions may be will only be determined by careful study of a large group of cases, analyzed from this point of view; the possibility that periarteritis nodosa may occasionally cause an acute exacerbation of previously existent rheumatic heart disease must be kept in mind. Assuming a rheumatic origin for a certain number of cases of periarteritis nodosa, it seems necessary, also, to assume that this type of vascular response is not specific in the sense of a single causative agent; the literature abounds with instances in which rheumatic fever can be definitely excluded as an etiologic factor. This is especially true of cases exhibiting the typical subcutaneous nodule of periarteritis nodosa, with which we are now concerned.

The incidence of subcutaneous nodules in the course of periarteritis nodosa has been variously estimated at from 15 per cent (198) to 25

per cent (199). The literature contains the reports of approximately 25 examples of the disease in which subdermic lesions were encountered, the latter presenting physical attributes vastly different from those shown by rheumatic lesions.

The outstanding fundamental feature of the nodule encountered in periarteritis nodosa is the origin from, and the definite relation to, an arterial branch that generally approximates the magnitude of a subcutaneous vessel; there is either thickening of the parietes or actual formation of a sacculated aneurysm. Ordinarily the nodules appear as small masses ranging in size from that of a pea to that of a hazelnut, giving rise to the impression of shotty thickenings along the course of an artery. Much skill and care must be exercised to find these structures and, for this reason, they may be easily overlooked by even the most experienced observer. Though the lesions are often superficially situated, it is a clinical feat to determine with certainty their arterial origin. The most common sites of aneurysmal formation in accessible arteries are the branches of the temporal, brachial, and intercostal vessels (200, 199). However, the arteries located in any part of the body may be affected, and it is generally the case that the lesions have an irregular distribution, contrasting in this respect with the regular sites of predilection shown by the rheumatic variety. It is possible, though not certain, that the examples of arteritis of the temporal vessels reported by several groups of observers (201, 202) belong in the category of periarteritis nodosa.

The number of nodules observed in a case of periarteritis nodosa may vary from a single lesion to as many as 15 or more. Sometimes there is a striking tendency to appear in groups along the course of one or several arteries. The nodules may be relatively soft and elastic at inception, but in their further course they characteristically become smaller and firmer in accordance with the nature of the pathologic alterations already enumerated. Rapid development is usual and the lesions may reach their definitive size in a short time. Regression may occur in several days, but this is variable; nodules may persist for over $1\frac{1}{2}$ years (203), the lesions in the interim becoming smaller and firmer. Complete disappearance in a clinical sense may occur, as in the case recorded by Manges and Baehr (199). Pain seems to be an inconstant feature; it may be outstanding or it may disappear

when the lesion reaches its maximum size, or it may be entirely absent throughout the course. In some cases there is redness of the overlying skin with accompanying tenderness, but even in the same crop of nodules this attribute varies. Sometimes there is adherence to the integument, the skin becoming thinned out, glistening, and eventually ulcerated. It appears that hemorrhagic extravasations may occasionally occur, the phenomenon being generally attributed to the rupture of a sacculated aneurysm, less commonly to the formation of a dissecting aneurysm (203). The rapid outpouring of blood elements may lead to breakdown of tissue with formation of necrotic ulcerations, often requiring considerable time for complete cicatrization. However, in some instances hemorrhagic extravasation is prevented by the striking tendency towards early thrombosis (199). For this reason, distinct pulsation is but rarely found. Matthes (204) observed an instance in which palpation of a nodule revealed pulsation synchronous with the apical beat of the heart. Fluctuating tumors have been encountered occasionally (203), but true suppuration is exceptional. In agreement with Klotz's opinion, it is likely that necrosis and liquefaction of fat, secondary to the nutritional disturbances imposed by the vascular changes, may give rise to an appearance simulating supuration; cultures of such material are usually sterile.

The initial clinical appearance of nodules may take place during the period of active progressive disease, although not infrequently they are first observed in the terminal stages (206, 199). There is no precise correspondence between the occurrence of nodular lesions internally and in the subcutaneous tissues. Cases have been described in which superficial lesions were diagnosed as periarteritis nodosa on the basis of microscopic changes in biopsied specimens, but in which post-mortem examination disclosed no evidence of this peculiar vascular reaction (207, 208); it would be wise to re-examine the status of such instances, a task beyond the scope of this article.

Finally, it must be stressed that the characteristic pathologic alterations of periarteritis nodosa appear to be restricted to the larger vessels of the subdermis and adjacent parts. Not all the circumscribed focal perivascular infiltrations seen in the dermis belong in the category of periarteritis nodosa, even when accompanied by hyalinization of the parietes and thrombus formation; similar changes, for example,

are encountered in typhus fever (209), a disease that probably bears no relation to periarteritis nodosa. Similarly, the changes in the walls of blood vessels found in erythema nodosum, in atypical examples of "arteritis", in Osler nodes, in Janeway lesions, in mycotic aneurysms etc. must be sharply differentiated from those of periarteritis nodosa. This subject is one bristling with complexities and sorely in need of elucidation.

SUMMARY

During the past few decades there has gradually arisen the doctrine that the subcutaneous nodule observed in rheumatic fever is pathognomonic of that disease as defined in its strict sense. The problem has, however, become complicated in several directions:

1. An attempt has been made to ally this manifestation with that found in rheumatoid arthritis. This has been based on certain clinico-pathologic evidence which is reviewed critically in the text. According to this hypothesis, the subcutaneous nodule is to be regarded as a link in the identification of rheumatic fever and rheumatoid arthritis under the broad category of the "rheumatic state".

2. The histologic changes seen in these lesions have been compared with the Aschoff body in the heart, some considering them as identical, others regarding the former as even more specific than the latter. These interpretations have been advanced chiefly by pathologists who have not subjected their views to the critique of clinical observation.

The data presented in this work have been buttressed by information gained from the following sources: (1) a study of a large number of various types of nodules that came under my observation and an analysis of a still greater number of clinical and postmortem protocols, over 500 and over 100 respectively. (2) An intensive investigation of the genuine rheumatic rashes and those encountered in clinically simulating conditions; the details of that work will be found in another publication. It may be stated that the conclusions arrived at in that study apply with equal force to the rheumatic subcutaneous nodule. (3) A fairly complete review and critical evaluation of the world's best literature on rheumatic fever and allied diseases.

The historical aspects were discussed in an effort to trace the de-

velopment of the conception of the rheumatic nodule and of resembling lesions. It was found essential to eliminate from the rheumatic category several types of lesions that were formerly included within its scope and that are now believed to be of different origin (Froberg's nodules, Jaccoud's nodules, Fereol's nodules, Heberden's nodes, miliary nodules of Coates and Thomas, Osler nodes and Janeway spots).

The genuine rheumatic subcutaneous nodule presents essentially the same attributes in all age groups. In adults, however, there is a greater tendency to show atypical localization, the incidence is much lower, and the diagnostic specificity is impaired to the extent that there are increased opportunities for confusing the condition with other diseases. It was therefore deemed advisable to discuss the lesion under two arbitrary headings: (a) in children (b) in adults.

The clinical features exhibited by the rheumatic nodules in childhood were described in their many aspects. This was followed by a comprehensive survey of the data bearing on the relations to rheumatic fever as defined in its strict sense. One of the most important questions discussed was that concerned with the incidence of heart disease; and evidence was presented to show that (1) the cardiac structure is "practically" always affected concurrently in one form or another; this feature is, however, the hall-mark of rheumatic fever itself, if not clinically, at least in postmortem examination; (2) that the occasional appearance of subcutaneous nodules in patients suffering from heart failure afforded evidence of continued rheumatic activity, the probable initiating or predisposing cause of the cardiac breakdown. Among other points mentioned were the relation to the true rheumatic eruptions and the failure of medication (salicylates and sulphanilimide) to prevent the onset of subcutaneous lesions. The question of prognosis was covered in great detail.

Following the description of the ordinary variants of the rheumatic subcutaneous nodule, an attempt was made to discuss critically some unusual appearances and their probable significance. The section on the lesions in childhood was concluded by a consideration of the differential diagnosis (subcutaneous nodules without heart disease; Still's disease; gouty tophi; granuloma annulare and erythema elevatum diutinum).

The same scheme was pursued in the account of the rheumatic

nodule in adults. The differential diagnosis was discussed under the following headings: rheumatoid arthritis, syphilis, gout, panniculitis and fibrositis, and, finally, periarteritis nodosa. Evidence was presented to show that the subcutaneous lesions found in these various conditions differed clinically from that in rheumatic fever and from one another. There was also included a detailed account of the pathologic alterations observed in these manifestations, and an effort was made to establish the principle that these nodules may reveal somewhat similar anatomical changes and that they were to be differentiated chiefly by their clinical attributes, the characteristic features of the individual diseases to which they owe their origin, and by the presence of "revealing substances," such as monosodium urate crystals. Particular attention was directed to the results obtained in certain experimental investigations. McEwen's supra-vital studies appear to merit the greatest consideration, as an attempt to differentiate the nodules in rheumatic fever and rheumatoid arthritis from those in other conditions. The final assessment of this method remains, however, to be determined.

Consideration of the data presented in this monograph seems to point in the direction of the establishment of the following principles:

1. The term rheumatic nodule should be applied to those lesions occurring in the course of undoubted rheumatic fever. The lesion presents definite characteristics, the most important being the location, the transiency (relative and absolute), and the relations to the other rheumatic phenomena, notably cardiac involvement. The highest degree of clinical specificity is enjoyed in childhood, and the rheumatic subcutaneous nodule may be said to be highly specific of rheumatic fever in that age group, provided the clinical attributes correspond closely with those mentioned in the text.

2. The specificity of the rheumatic nodule is dependent on its clinical properties and relations. The combination of pathologic changes found in microscopic examinations, while often suggestive, shows no pathognomonic characteristics and may be simulated by other lesions.

3. The true rheumatic nodule is practically always associated with clinical evidence of cardiac involvement in one form or another. The evidence compiled in this monograph substantiates the view that disease of the heart is the fundamental hall-mark of rheumatic fever.

This may not always be evident clinically, but its occurrence is to be expected at postmortem examination. When evidence of cardiac involvement in one form or another is completely lacking at necropsy, there is reason to believe that the case was not one of rheumatic fever and the burden of proof rests on those who wish to classify the condition in the rheumatic category. On the other hand, the presence of rheumatic heart disease, particularly if the changes are old and healed, does not necessarily indicate that nodules appearing at the time are inevitably related to the rheumatic process; each case must, therefore, be evaluated critically, and the factor of coincidence must be taken into account. The difficulties inherent in this problem are increased owing to the lack of uniformity in the pathologic criteria for the recognition of rheumatic fever.

4. The typical nodule in rheumatoid arthritis differs from that in rheumatic fever in many clinical attributes and in some pathologic respects. The clinical differences appear to be more important than the pathologic differences, the latter still requiring evaluation.

5. The nodule in rheumatoid arthritis shows greater resemblances to the juxta-articular node in syphilis. This is especially true in a clinical sense and it probably also holds pathologically, if the ordinary methods of staining are used for microscopic study. On the other hand, it is possible that investigations pursued by supra-vital staining (McEwen) may furnish data of differential diagnostic importance.

6. The conception of the syphilitic nodule as an entity rests on three features: (1) its association with other manifestations of syphilis; (2) the almost invariable presence of a positive Wassermann reaction; (3) the striking response to anti-syphilitic remedies.

7. The controversy regarding the relative incidence of subcutaneous lesions in rheumatoid arthritis and in syphilis is clarified by the realization that both varieties of nodules occur, but that their respective incidence will be governed largely by the type of material under observation.

8. The pathologic criteria for the diagnosis of a "rheumatic nodule" are discussed critically. Evidence is presented to show that these appearances, as observed in the ordinary microscopic examinations, are not pathognomonic of a single disease. How far the supra-vital studies will provide criteria for the differentiation of the various nod-

ules is still problematic, but it is a method worthy of extended investigation. Caution is advised in drawing etiologic conclusions on the basis of morphologic resemblances.

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THE COAGULATION OF THE BLOOD

P. NOLF

From the Fondation Médicale Reine Elisabeth, Brussels, Belgium

H. Eagle, in an article published a year ago in *MEDICINE* (1) describing the recent advances in the blood coagulation problem, appreciated in the following terms my personal explanation of coagulation:

"Thus, Nolf suggested that fibrinogen, a hypothetical protein 'thrombogen,' and 'thrombozyme,' a hypothetical enzyme derived from white blood cells and the walls of the blood vessels, interact to form fibrin and thrombin, and that thrombin is a *result* of coagulation rather than its cause. His theory includes numerous bizarre relationships between the reagents for which later investigators can find no confirmation."

I think I am entitled to consider this sentence passed on the results of the research work of many years, as somewhat summary; the more so, that the said results do not consist exclusively of hypotheses.

I have therefore proposed to the editor of this review that he allow me to draw up for his readers, as objective a statement as possible of the results of my observations and of the reasons why I still feel unable, even after H. Eagle's article, to rally to what might be called the classic, or current theory of blood coagulation. And I am all the more tempted to write this justification of my opinion because most of the authors who have taken a stand against it, have proved, by their inaccurate rendering of it, that they have not read my original papers and have no direct knowledge of the actual experimental data on which my interpretation is based.

THE PART OF THE LIVER IN BLOOD COAGULATION

I began my study of blood clotting by experimenting on living animals. In 1904, Doyon and Kareff (2) published, in the *Comptes-Rendus de la Société de Biologie*, a brief communication on the effect of the removal of the liver on the coagulation of blood. The experi-

ment had been made on one dog only, the lobes of whose liver they had excised in fragments, thereafter joining by means of a glass canula, the portal vein to one of the hepatic veins. The blood of the animal had become incoagulable before its death. In their first note, the authors suggested no explanation for this incoagulability. Before them, Gley and Pachon in 1895, Hedon and Delezenne in 1896, Nencki and Pawlow in 1897, Saleskin and Zaleski in 1899 had extirpated the liver in dogs without noting any appreciable difference in the coagulability of their blood. I had myself, prior to Doyon and Kareff, published the results of experiments on dogs, in which, after a preliminary ligature of both the internal mammary arteries at their origin, the aorta and the inferior vena cava were clamped above the diaphragm in such a manner as to limit the blood circulation to the upper part of the body (3). After several hours, the coagulability of their blood showed no appreciable signs of disturbance. If an injection of peptone was made in the jugular vein, it did not cause incoagulability, as it does in the normal animal; the blood clotted in the normal lapse of time, or even in a shorter time, but the clot dissolved after one or two days, so that the blood became completely fluid again.

These results did not agree with those published by Doyon and Kareff, and I was consequently lead to repeat their experiment. Two objections could be raised against their technique. On the one hand, the liver had not been extirpated entirely; on the other, the use of a glass tube to anastomose the portal vein with the vena cava brought the circulating blood in contact with a foreign body which it wets. And it is a known fact that such foreign bodies exercise a coagulating influence on blood, even when it is circulating in the vessels of a living animal.

Another technique had to be found to remove the liver in such a way as to avoid the shortcomings of that used by Doyon and Kareff. A glass canula, or rather two, were used, but after they had first been carefully lined with a continuous layer of paraffine. One of them established direct communication between the main trunk of the portal vein and the right auricle, the other between the inferior vena cava below the liver and the same vein above the liver. Once the two canulae have been placed, the liver may be completely removed without the slightest difficulty (4). The main objection to this technique

lies in the fact that it requires the opening of the thorax and artificial respiration. At the time of the death of the animal, the inner parafined surface of the canulae is usually entirely smooth. No parietal deposit of fibrin is to be found on it. In the series of experiments published in 1904, the animals survived, on an average, two hours, and never more than two and a half hours. At the moment of death, the blood coagulated within the normal lapse of time and showed no visible signs of disturbance in the clotting process. Results just as negative were obtained by Mann, Bolmann and Markowitz in 1929 in animals whose liver had been extirpated according to a technique far superior to that which I had used in 1904, and which allowed the animals to survive for more than one full day (5).

It is therefore not true to say that the extirpation of the liver in dogs alone suffices to prevent the blood from clotting.

But if, after the liver has been removed, an intravenous injection of Witte peptone is made (0.20 to 1 gram per kilogram), the clotting phenomenon immediately undergoes very interesting changes. A sample of carotid blood, drawn immediately after the injection, coagulates in a few minutes; but the clot, firm at the beginning, undergoes afterwards a process of fibrinolysis, so that, after less than 24 hours at room temperature, it is completely redissolved and the blood is entirely fluid again. This tendency to fibrinolysis increases gradually in each of the blood samples which are drawn in succession until the animal dies. The blood clot dissolves in a few hours at room temperature and more rapidly still at 37°C. Concomitantly, the time of clotting lengthens and the clots are increasingly softer and more incomplete. Sometimes the blood is even incoagulable in the sense that it only clots partially on contact with glass, the greater mass of the blood remaining fluid. These incomplete blood clots, furthermore, undergo their secondary dissolution very early.

These profound changes in the blood clotting process are due to two factors. The first is the progressive disappearance of fibrinogen which can be demonstrated in the following manner. When blood is drawn to measure clotting time, a part of it is mixed into 1/10th its bulk of a 3 per cent solution of sodium fluoride, or of a 1 per cent solution of sodium oxalate, so as to prevent clotting. The fluoride, or oxalate plasma is separated from the blood corpuscles, and serves

for determining its content in fibrinogen. The measurements show a rapid fall in the quantity of fibrinogen. In certain animals, immediately before their death, that is to say about two hours after the liver has been extirpated and peptone injected, fibrinogen may even end by disappearing completely.

The disappearance of fibrinogen explains the incoagulability of the blood of these animals, but it does not explain the other change in the blood clot: its great tendency to fibrinolysis. The fall in the fibrinogen content probably promotes fibrinolysis, but the main reason for this latter process must be sought in the disappearance of another substance which also plays a part in blood-clotting and whose function is to prevent fibrinolysis. This substance exists in normal plasma. For if, before it clots, the fibrinolytic blood is mixed with 1/60th of its bulk of normal dog plasma, the clot will not dissolve. The normal plasma does not act through the agency of the trifling quantity of fibrinogen it carries, for it remains active after heating at 56°. The antifibrinolytic action is due to a different protein than fibrinogen, which is also produced in the liver.

This was proved later in dogs whose circulation is restricted to the upper part of the body, above the diaphragm (6). A sufficiently long part of the aorta is freed immediately below the diaphragm, cut transversally and anastomosed by inosculation to the upper end of the cut portal vein. The vena cava is ligatured immediately under the liver. In this way, the liver is irrigated directly by the aorta and it is the only organ below the diaphragm provided with circulating blood. When the animal has recovered from the operation, the liver is momentarily excluded from circulation, the aorta being clamped below the liver and the inferior vena cava above the liver. Immediately after occlusion, peptone is injected into the jugular vein. Blood drawn from a carotid artery two minutes after the injection clots within a normal, or even possibly a shorter lapse of time, and the clot regularly undergoes complete fibrinolysis after one or two days at ordinary temperature. This fibrinolytic property can be suppressed merely by opening the aorta and the vena cava up again and reinstating circulation through the liver. But one should, in order to obtain a decisive result, avoid one cause of complication. If the blood vessels are opened up too swiftly, that is to say, less than ten minutes after peptone has been

injected, the blood drawn after the disocclusion is both incoagulable and anti-coagulant. Mixed in small quantities with the blood of a normal animal, it prevents that blood from clotting. It behaves like the blood from a normal dog intravenously injected with peptone. The anticoagulant property discovered by Schmidt-Mülheim in Ludwig's laboratory was attributed by Fano (1881) to a substance contained in the plasma and destroyed at 100° , which he called antithrombin. I thought antithrombosin a more adequate term because it only asserts the anticoagulant property. It was later shown by a series of French authors (Contejean, Gley, Hedon and Delezenne) that it is produced by the liver. The preceding experiment confirms anew the hepatic origin of antithrombosin. The production of antithrombosin can be prevented by interrupting the circulation in the liver for a given lapse of time before the blood of the upper part of the body is allowed to flow through it again. An interruption of ten minutes is enough to secure this result, the injection of peptone having been made in the jugular vein immediately after the occlusion. Under these conditions, the blood no longer becomes incoagulable after the re-establishment of circulation, but it recovers its normal qualities, that is to say, the clot no longer undergoes fibrinolysis. Since it is enough to mix a small quantity of normal plasma with the blood of an animal deprived of its liver and injected with peptone to prevent fibrinolysis, it may be asked whether the disappearance of fibrinolysis in the above experiment should be attributed to the action of the liver, or simply to the mingling of the blood contained in the liver with that circulating above the diaphragm. This latter explanation may be discarded for the following reason: several samples of blood are drawn successively after the re-establishment of circulation in the liver. Return to normality occurs gradually. In fact, a completely normal clotting is only achieved after several minutes, which necessarily implies the active intervention of the liver in the production of the antifibrinolytic principle.

Just like antithrombosin, the antifibrinolytic substance is non-diffusible and it is coagulated by heat; both are proteins, and normally present in the plasma of circulating blood.

The facts just recorded, and others which it would be too long to describe here, lead to the conclusion that the liver produces the three

proteic substances, fibrinogen, antithrombosin and the antifibrinolytic factor, and that all three play a part in blood clotting. This is confirmed by the influence exercised on blood coagulation by severe lesions of the liver caused by poisons such as phosphorus or chloroform. Corin and Anciaux were the first to study the effects of phosphorus intoxication on blood clotting in the dog (7). They noted in the intoxicated animal concomitant disappearance of fibrinogen and of prothrombin. If the blood of the said animals becomes incoagulable, it is because it loses simultaneously what constitutes, according to the generally accepted theory, the two essential factors of coagulation. Morawitz ultimately confirmed the observations reported by Corin and Anciaux. Doyon (8), Whipple and Hurwitz (9) and recently Smith, Warner and Brinkhouse (10) came to similar conclusions as regards the action of chloroform.

In a dog dying after several days of sub-acute phosphorus intoxication, the blood drawn immediately before the animal's death is usually incoagulable. The plasma, separated from the blood corpuscles, habitually leaves a few fibrinous shreds. The supernatant fluid, in certain cases, coagulates the fibrinogen solution. At ordinary temperature, the clot redissolves after a few hours. The plasma is often devoid of any obvious coagulant action on fibrinogen. If to the mixture of this plasma and fibrinogen, a minute quantity of oxalate dog plasma previously heated at 56°C. is added, in the presence of an adequate quantity of calcium salts, a clot is formed which does not ultimately undergo fibrinolysis (P. Nolf, (11)). The mixture of fibrinogen and of plasma from the phosphor-intoxicated animal behaves, in these experiments, like the blood of a dog which, after extirpation of its liver, has received an intravenous injection of peptone. Even if this blood were not deprived of its fibrinogen, it would still fail to coagulate, or would do so incompletely, because it not only lacks fibrinogen, but also contains an inadequate quantity of the antifibrinolytic agent also necessary for blood clotting, which will be called thrombogen.

These studies of the changes in the clotting process of the blood of dogs whose liver has been extirpated, or severely injured by intoxication, lead to the conclusion that three factors at least take part in the coagulation of blood, and that these factors are present in the plasma. Two, thrombogen and fibrinogen, are produced by the liver. The

third, which has been called thrombozym (P. Nolf), is found abundantly in the plasma of dogs intoxicated by phosphorus or chloroform. It is also predominant in the blood of dogs whose liver has been extirpated and which have been injected with peptone. Thrombozym does not coagulate pure fibrinogen; it coagulates only a mixture of thrombogen and fibrinogen; it is also, when prevailing, responsible for the redissolution of the clot.

Thrombozym is not of hepatic origin. A series of facts, too long to be put down here, allow one to assert that it is produced by certain white blood cells and by the endothelium of the capillaries.

BLOOD COAGULATION IN VITRO

The above-mentioned notions derived from the observation of the living animal tally with the facts observed in blood clotting in vitro. According to the schema of A. Schmidt (last version), to which most of the authors interested in blood coagulation remain true to this day, the plasma of circulating blood is deprived of an element necessary to form thrombin. That is why it does not clot in the vessels. After blood has been shed, the platelets (or the nucleated thrombocytes in the oviparous vertebrates), irritated or altered through contact with foreign bodies, release the coagulant substance they contain. In the presence of soluble calcium salts, the latter reacts with the precursor of thrombin, which is present in the plasma and is usually called prothrombin, and turns it into thrombin (1st phase); thrombin then changes fibrinogen into fibrin (2nd phase). Though generally admitted, this explanation does not agree with the well established fact that every natural plasma not only contains all that it needs to clot, but also to produce a large excess of thrombin.

It is possible to collect blood from oviparous vertebrates, fish, batrachians, reptiles and birds, in such a manner that it remains indefinitely fluid, without the addition of any foreign substances, merely by drawing it through a paraffined canula into a paraffined vessel. After a first rapid centrifugalisation, the plasma is decanted with a paraffined pipette, then centrifuged anew in a paraffined vessel until it is absolutely clear and no longer deposits any cells. This plasma may then be considered as absolutely cell-free. If a sample of it is kept in a clean glass tube, which has not been lined with paraffine,

coagulation is kept in abeyance indefinitely at ordinary laboratory temperature. But if a small quantity of aqueous, or even alcoholic extract of white blood cells, or of any other tissue, is added, it clots immediately. This is the main argument used in favour of A. Schmidt's theory. It would be clinching were it proved that clotting of cell-free plasma is produced by tissue extracts only. This is not so. It has been shown that all these cell-free plasmas of oviparous vertebrates respond to the coagulant action of a great many substances which do not exist in cellular extracts, and which can be asserted not to have any part in spontaneous blood clotting. Of these substances, the one most studied is chloroform (12). Shaken with one-twentieth its volume of chloroform, bird plasma coagulates after about ten minutes. Having been separated from the fibrin, the serum is put in watch-glasses until the chloroform has evaporated completely at 0°C. After having been brought back to its initial volume by the addition of distilled water, the serum is very active. Even in small doses, it coagulates fresh bird plasma in a short time, and just as easily coagulates an oxalated solution of fibrinogen. Its coagulant action must undoubtedly be attributed to thrombin. Consequently, a clotting of the plasma has occurred, with fibrin formation, under the influence of chloroform, whereas an enormous excess of thrombin appears in the serum.

If two samples of the same bird plasma are taken, the one being clotted by chloroform, the other by alcoholic tissue extract, it may be found that *the first serum contains 10 to 30 times more thrombin than the second*. Compared with the serum left after the spontaneous coagulation of the whole blood of the same bird, its thrombin content is 30 to 100 times higher. According to A. Schmidt's theory, the thrombin contained in the serum of the whole blood is a resultant of the action on the prothrombin of the plasma of substances released by the white blood cells. Thus then these said substances are only capable of producing 30 to 100 times less thrombin than is produced under the influence of chloroform at the expense of the elements contained in the plasma.

Given these results, it is impossible to go on asserting that a completely cell-free plasma does not contain all the elements required for the formation of thrombin. It contains them overabundantly.

The blood of mammals differs from that of birds in that its tendency to spontaneous coagulation is far more marked. This difference is not due, contrary to the current view (J. Bordet), to the presence in the blood of mammals of platelets which, are absent from the blood of oviparous vertebrates, but it is inherent to the plasma. For the lymph of a dog collected from a lymphatic of the neck or of a limb, which does not carry any platelets, is just as coagulable as its blood (30).

It is on account of this greater tendency to coagulation that the preparation of a cell-free plasma is rendered all the more difficult. The simplest method is to receive the blood, as it flows out of the artery, into the required volume of a solution of sodium fluoride, oxalate or citrate. Separated by centrifugalisation, the cell-free plasma coagulates on recalcification. But it could be objected that mixing the blood with the aforesaid saline solutions has altered the white cells and platelets and has made them liberate their coagulant factor. This objection can be avoided by using paraffined vessels for drawing and centrifuging the blood. Received through a paraffined canula into a paraffined vessel, the blood of mammals does not clot, and the plasma can be separated through centrifugalisation without any difficulty. Decanted into a paraffined tube, the plasma remains fluid at room temperature. Decanted into a non-paraffined tube, it clots. And yet no cellular elements are suspended in it (J. Bordet).

Another method for preparing the cell-free plasma of mammals can be used, when the animal is large enough (dog, horse, etc.): a segment of the jugular vein is isolated between two ligatures, excised and placed into a centrifuging tube. Thus the blood is subjected to the action of centrifugal force without being taken out of the vein. When the corpuscles have been completely separated, the vein is ligatured above the layer of platelets and the plasma is drawn from the segment above the ligature, the first drops being discarded as being possibly tainted with a little tissue juice (P. Nolf, 1904) (4). This plasma fails to clot in a paraffined tube, but does clot in an ordinary glass tube. We here witness the coagulant action of the contact with glass in the absence of any cellular element.

Instead of using centrifugalisation to separate cells from plasma, one may also use filtration through a living membrane, such for

instance as the ciliary processes which filter the aqueous humour. If the cornea is punctured, in a big dog, with a thin, sharpened glass tube, the first few drops of aqueous humour are deprived of protein and do not coagulate. Then, intra-ocular pressure having disappeared, a newly formed aqueous humour filters through the ciliary processes and drops from the glass tube which has remained fixed in the cornea. Like the normal, this second aqueous humour is absolutely crystal clear and no cells whatever are suspended in it. Yet, like the blood- or lymphplasma it coagulates on contact with glass in a relatively short time.

Any author who should wish to do these experiments again, will have no difficulty in ascertaining the coagulability of the cell-free blood-, lymphplasma, or aqueous humour of mammals. How can any one, therefore, go on pretending that the plasma of vertebrates lacks one of the elements requisite for clotting, and that this element is produced by the white blood cells at the moment of extravasation? The main argument based on actual facts was brought forth by Cramer and Pringle. The experiment consists in passing oxalate plasma through a Berkefeld filter and subsequently adding calcium to it. Before filtration, it clots on recalcification. After filtration, it no longer clots. The authors explain this result by saying that filtration has kept back platelets, or fragments of platelets, which were suspended in the plasma. For if they add platelets to the filtered plasma, the latter again clots on recalcification. Cramer and Pringle's explanation would be valid if they could prove that filtration does not deprive the plasma of any dissolved element. But it has been known for a long time that the haemolytic complement, beyond a doubt dissolved in the serum, does not pass through a Berkefeld filter. The same holds true for a fair quantity of the fibrinogen and of the mother-substances of thrombin (P. Nolf).

Another argument brought up by the partisans of A. Schmidt's theory is that the clotting of the cell-free plasma of mammals is far slower than that of the whole blood, and that the resulting serum is not so rich in thrombin.

The experiments quoted above, concerning the cell-free plasma of oviparous vertebrates, have shown that a plasma may have no tendency to spontaneous coagulation, even when in contact with glass, and

yet be overabundantly provided with all the elements required for the formation of fibrin and of a considerable excess of thrombin. This is even truer of the plasma of mammals than of the plasma of birds. A cell-free horse plasma, under the influence of chloroform, produces great quantities of thrombin; so also does citrated rabbit plasma which has been passed through a Berkefeld filter and recalcified afterwards. (P. Bordet) (13). If the plasma has all the elements needed for clotting, and yet fails to clot in the vessels, it is for the simple reason that it contains, in a constant manner, though in concentrations varying according to species, the proteic substance produced in the liver and called antithrombosin. Before investigating the mode of action of the latter substance, it is advisable to ascertain first the chemical properties of the coagulant agents, the existence of which has been proved previously, and the part they play in the coagulating process.

MOTHER-SUBSTANCES OF THROMBIN AND FIBRIN

Crude thrombozyme was, for the first time, separated from the plasma by Wooldridge in 1884 and called A-Fibrinogen (14). He used the cell-free plasma of a dog whose blood had been rendered incoagulable by an intravenous injection of peptone. This plasma, henceforth called peptone plasma, does not clot spontaneously even on contact with glass; as has been said before, it even prevents the coagulation of normal blood on being added to it. And yet it can be made to clot without any addition of tissue extract, simply by diluting it with distilled water and bubbling CO_2 through it. As Wooldridge has observed, simultaneously with the clotting, there appears in the serum an appreciable quantity of thrombin.

If a non-diluted and absolutely clear plasma is kept at 0°C ., it becomes cloudy and, after 24 hours, deposits a very light precipitate; under the microscope, the latter seems made up of thin, transparent discs whose dimensions are approximatively those of blood platelets. It is this precipitate which Wooldridge called A-Fibrinogen. He considered it an agent in the clotting of the plasma, because a peptone plasma, freed of this precipitate by filtration at 0°C ., no longer coagulates when CO_2 is bubbled through it after dilution with distilled water. This substance, discovered by Wooldridge, does not seem to have retained the attention of other physiologists. Hammarsten

alone says that oxalate horse plasma, kept for several days at $0^{\circ}\text{C}.$, habitually becomes cloudy (15). If it is freed by filtration at $0^{\circ}\text{C}.$ of its precipitate, it reacts less completely than the original plasma to the coagulant action of added calcium salts. Most of the samples of oxalate horse plasma behave in the manner described by Hammarsten. But as, in many cases, the plasma must be kept at $0^{\circ}\text{C}.$ several days before it precipitates, the proof must be brought that the lesser coagulability of the plasma is caused by the separation of the precipitate, and not by an alteration of the plasma due to its having been kept so long.

It is easy to demonstrate the truth of the first alternative by redissolving the precipitate in a certain quantity of the plasma; at $37^{\circ}\text{C}.$ the coagulability of the latter is completely restored and even increased according to the quantity which has been redissolved.

The substance which Wooldridge termed A-Fibrinogen, can be isolated from horse plasma and purified by several redissolutions at $37^{\circ}\text{C}.$ and reprecipitations at $0^{\circ}\text{C}.$ (P. Nolf) (16). In the presence of calcium salts, this purified A-Fibrinogen does not coagulate a pure solution of fibrinogen. Therefore it cannot be said to be prothrombin. It does clot a plasma which two or three successive additions of sodium oxalate 0.5 per cent and subsequent recalcification have made spontaneously incoagulable. This treatment partly deprives the plasma of its A-Fibrinogen which adheres to the bulky precipitate of calcium oxalate, but leaves it in possession of nearly all its thrombogen and fibrinogen. Wooldridge's substance also clots a solution of fibrinogen to which has been added a trifling quantity (1/50th of its bulk) of the aforesaid plasma (which does not coagulate the fibrinogen when used alone). The clot resulting from the last mentioned mixture autolyses, when it contains a sufficient amount of the purified substance. The coagulant and fibrinolytic properties of this substance can also be shown by adding an adequate quantity of it to oxalate horse plasma (0.15 per cent) diluted with nine volumes of an isotonic sodium chloride solution provided with calcium. The formation of the clot is hastened and the latter redissolves entirely, whereas the clot formed from the pure diluted and recalcified oxalate horse plasma does not autolyse. Through its being both coagulant and proteolysing, Wooldridge's A-Fibrinogen identifies itself with thrombozym.

Cooling of the oxalate plasma at 0°C. only makes a small fraction of the thrombozym precipitate, whereas the greater part remains dissolved. Therefore the plasma is still coagulable by recalcification, although to a lesser degree. Addition of sodium oxalate 0.5 per cent and subsequent recalcification make it still more uncoagulable, but also fail to separate the thrombozym completely. There is actually no known method to achieve this result, which does not interfere with the thrombogen and the fibrinogen.

The above mentioned facts relating to blood clotting *in vitro* are similar to those which occur in the dog after extirpation of its liver and injection of peptone, and which have been stated previously. In both series, coagulation prepares the redissolution of the clot; in both, coagulation and fibrinolysis result from the reaction on each other of three substances present in the plasma.

Simultaneously with the redissolution of the clot, there occurs a change in the fibrinogen: previously to its clotting, it is endowed with the property of being coagulable through heating at 56°C. Thus, the oxalate plasma collected from a dog which has just been deprived of its liver and injected with peptone, becomes cloudy after heating at 56°C. and deposits a light precipitate of coagulated fibrinogen. But if the blood is allowed to clot, and, after fibrinolysis has taken place, the blood corpuscles are separated from the supernatant fluid, the latter, when heated at 56°C., no longer becomes cloudy nor deposits any precipitate: the fibrinogen has begun to go through a process of proteolysis. The same change is observed *in vitro*, when the clots rich in thrombozym have suffered fibrinolysis. After having thus rendered the fibrinogen more soluble, thrombozym proteolyses it further with production of proteoses. Since the agent of this proteolysis, thrombozym, was isolated from oxalate horse plasma, it is somewhat strange that all these facts should be summed up in H. Eagle's article in one brief reference to a "hypothetical proteolytic enzyme." The more so that this author has devoted special attention to the coagulant action of other proteolytic ferments, such as trypsin and papain. It does not seem very probable that either the one or the other should have any bearing on normal blood clotting, whereas the reverse is true of thrombozym, which was isolated from the normal plasma of mammals and birds.

COAGULATION AND FIBRINOLYSIS

In my opinion, the clotting of the plasma is a preliminary step towards a proteolysis. The proteolytic ferment, thrombozym, becomes insoluble together with two proteic substances of the plasma: thrombogen and fibrinogen, in order to make fibrin. The subsequent fate of the clot depends on its content in thrombozym and thrombogen. If there is an excess of the first, the fibrin undergoes a prompt proteolysis, the first token of which is its redissolution. If, on the contrary, thrombogen is dominant, fibrin withstands proteolysis more or less long, sometimes even indefinitely. The latter alternative corresponds to the usual clotting conditions in the bird or mammal.

A great many facts can be adduced in support of the opinion that the coagulation of fibrinogen and its subsequent proteolysis are not two distinct processes, but should be considered as two successive phases of one and the same phenomenon (17). Some of these facts are related to the coagulation of pure fibrinogen by thrombin which, as will be shown further, results from the union of thrombozym with thrombogen. The mixture in an oxalated medium of pure fibrinogen with pure thrombin, prepared according to A. Schmidt, is the simplest which can be used in experiments on coagulation. The fibrin resulting from its clotting regularly redissolves completely after standing more or less long at 37°C. on condition that the solution of fibrinogen be sufficiently diluted (0.1 to 0.3 per thousand).

Instead of a solution of thrombin prepared according to A. Schmidt, another may be used which is made by dissolving pig fibrin in sodium chloride at 10 per cent, or in isotonic saline saturated with chloroform or toluene, or in a solution of 1 per cent of sodium fluoride. For as much as the fibrin has been left in contact at 37°C. for one or two days with these liquids, the resulting solutions clot the diluted fibrinogen energetically, and the clots subsequently redissolve completely in the medium in which they were produced. At first sight, the clots obtained by adding fresh serum of mammals to the same solution of fibrinogen, have a different fate. They do not, apparently, show the same tendency to fibrinolysis. But for being less obvious under the usual conditions, the said tendency exists nevertheless. It can be shown that the fate of the clot depends essentially on the proportion of fresh serum to the volume of the diluted solution of

fibrinogen. The experiment may be carried out with fresh dog serum. Added in the proportion of 1/10th of the bulk of the solution of fibrinogen, it produces a clot which does not fibrinolyse. But if the volume of the serum drops to about a 1/500 to 1/15000th part of the fibrinogen solution, the clot, which is formed all the more slowly that the amount of serum used is less, redissolves at a rate which is inversely proportional to the clotting time. Things happen as if the difference between the serum and the fibrin solution does not lie in the nature of the coagulant agent. In both cases, the latter apparently causes successively coagulation and fibrinolysis, but besides it, a substance is present in the serum which prevents fibrinolysis; its antifibrinolytic action is weakened by dilution at a swifter rate than the coagulant activity of thrombin.

From the fact that there is no solution of thrombin which does not at the same time contain the fibrinolytic agent, it has been concluded that thrombin is itself endowed with the fibrinolytic property which it owes to its content in thrombozym. The following experiment may be quoted in support of this opinion (17).

An emulsion of lymphocytes is prepared from mesenteric lymph glands taken from a dog whose hindquarters, after death, have been deprived of all their blood by an intravascular injection of several litres of isotonic saline. These glands are crushed in isotonic salt solution and the emulsion thus obtained is filtered through fine gauze and centrifuged in order to get rid of the supernatant liquid. The lymphocytes having been washed once more with isotonic salt water at low temperature, are suspended in a small volume of isotonic saline so as to make a moderately opaque emulsion. When it is added to a diluted solution of fibrinogen, in the presence of the requisite quantity of calcium salts, the lymphocytes emulsion does not produce coagulation. If to this same mixture of fibrinogen and of lymphocytes is added a certain quantity of oxalate or fluoride dog plasma (varying between 1/250 and 1/1000th of the total volume), which has been heated previously at 56°C. and does not clot the fibrinogen by itself, this mixture (A) coagulates in a few minutes and the clot, kept at 37°C., is redissolved after two to three hours. In a control, these same reagents are mingled in the same proportions, but instead of the calcium salts, sodium oxalate is used in the proportion of 1/1000 (mix-

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ture B). Mixtures A and B are prepared simultaneously and put at 37°C. When the clot in mixture A is entirely dissolved, calcium chloride is added to mixture B so as to neutralize the sodium oxalate and make coagulation possible. The medium which had remained completely fluid until recalcification, is put once more at 37°C. It clots, and the clot dissolves subsequently. Oxalate has thus prevented the proteolysis of fibrinogen as well as its coagulation. If, simultaneously with mixtures A and B, a third mixture is put at 37°C., containing the same quantity of fibrinogen, of lymphocytes and of calcium as mixture A, but no plasma, this mixture is still fluid when mixture A, after having first clotted, has undergone total fibrinolysis. If the small quantity of plasma contained in A and B is then added to C, both the clotting and the subsequent fibrinolysis occur. In this latter experiment the lymphocytes fail to proteolyse fibrinogen, as long as they have not clotted it.

From these experiments it has been concluded that clotting is an obligatory preliminary step towards the proteolysis of fibrinogen by the agent contained in the lymphocytes. This conclusion is corroborated by the results of experiments on the clotting action of chloroform on cell-free plasma.

As has been said above, chloroform emulsified by vigorous shaking with bird plasma for several minutes coagulates it in a short time; the serum given off by the clot contains large quantities of thrombin. Whereas the clot from whole bird blood or from pure plasma does not show the slightest tendency to fibrinolysis, the clot formed from cell-free plasma under the influence of chloroform redissolves in its own serum after a few hours and its dissolution is, as usual, accompanied by the proteolysis of fibrinogen. A control specimen of the plasma which has been kept at the same temperature, but not mixed with chloroform, is still fluid and coagulable. Here again, the clotting of fibrinogen appears as the unavoidable preliminary to proteolysis (12).

This conception of the relation between the clotting of fibrinogen and its proteolysis by one and the same agent, is wholly different from A. Schmidt's. For him, the transformation by thrombin of fibrinogen into fibrin is in itself a proteolysis in the sense that: "thrombin splits fibrinogen to form fibrin." From my point of view, the clotting of fibrinogen by thrombin does not identify itself with proteolysis, but

precedes it. In other words, fibrin is not the outcome of a "splitting of the fibrinogen," but the product of the union of thrombin with fibrinogen. It is only subsequent to its union with fibrinogen that thrombin proteolyses the latter.

According to my interpretation of the clotting of plasma, fibrin is formed by the union of three substances: thrombozym, thrombogen and fibrinogen, which are present in the cell-free plasma of all vertebrates. Of these three substances, the two first exceed the third to a considerable extent, in terms of equivalence. When the two first are found together in a medium containing calcium salts, they are capable of uniting to form thrombin even in the absence of the third, fibrinogen, as I was first to establish in 1908 (18). Thrombin is the result of their union, because its formation goes hand in hand with the consumption of thrombozym and thrombogen. When a cell-free plasma clots, the reaction between thrombozym and thrombogen is accelerated by fibrinogen, which takes up the thrombin as fast as it is formed, so as to make with it an insoluble compound, fibrin (18). As soon as all of the fibrinogen is used up, its accelerating influence comes to an end, together with the reaction between thrombozym and thrombogen. This explains why the serum resulting from the spontaneous clotting of the cell-free plasma of vertebrates usually contains but small quantities of thrombin (mammals), or traces (birds), or none at all (fishes). Yet it always holds a very notable excess of thrombozym and of thrombogen in a free state. But if this serum is shaken with chloroform, the residual thrombozym and thrombogen will unite to form a very large quantity of new thrombin (P. Nolf, 1921).

The serum obtained by the action of chloroform either on the cell-free plasma itself, or on the serum given off by a previous spontaneous clotting of the said plasma, undergoes very curious changes in its coagulant properties, if it is digested in contact with chloroform for several days at room temperature. These changes are essentially alike in birds (fowl, duck) and in dogs, but particularly plain in the latter (19, 20), as is brought out by the following experiment: Recalcified oxalate dog plasma is clotted by chloroform; after evaporation of the latter at 0°C., the serum coagulates an oxalated solution of fibrinogen very actively. The clots, however, differ from those obtained from normal blood serum in that they show a greater tend-

ency to spontaneous fibrinolysis. If the plasma, after having been clotted by the chloroform, is left for a few hours in contact with this liquid at room temperature, the fibrinolytic property of the serum becomes even more marked. The clots produced by this serum, when it is added to a solution of fibrinogen, have but a brief existence: within a few hours, sometimes a few minutes, they are completely redissolved. After a still longer digestion in contact with chloroform, there comes a time when the serum (the chloroform having first been removed) becomes unable to clot the fibrinogen solution. No sooner has it been added to the latter than the mixture becomes opalescent, owing to the formation of aggregates resulting from the union of the thrombin of the digested serum with fibrinogen. These compounds are not insoluble like those issuing from the union of normal thrombin with fibrinogen. They remain in solution, but undergo an extremely swift autolysis, as results from the following fact. The mixture of the digested serum and of fibrinogen loses within a few minutes its property of being coagulated by such a quantity of normal thrombin as would suffice to clot quickly the same volume of a solution of pure fibrinogen. Under the influence of the digested serum the fibrinogen has become uncoagulable; it has been proteolysed. During the prolonged digestion of the serum in contact with chloroform, the thrombin has thus been autolysed so as to lose the power of clotting fibrinogen, but to retain that of proteolysing it. It behaves like trypsin, and no longer like ordinary thrombin.

If it fails to clot pure fibrinogen, this autolysed thrombin is, however, just like snake venom, staphylotoxin or trypsin, still able to clot actively normal plasma, and even oxalate plasma. The resulting clot does not redissolve, provided that the added doses of serum are not too strong.

Here again is the evidence for the presence in the plasma of a substance which plays a twofold part in the coagulation of blood: (1) Without it, the autolysed thrombin is unable to turn fibrinogen into insoluble fibrin; (2) it counteracts the proteolysis of fibrin. This substance is found in the plasma of any vertebrate and is absent from a solution of pure fibrinogen; it is none other than thrombogen.

In order to grasp the nature of the fundamental changes wrought by chloroform on the coagulant property of thrombin, when it is left

in contact with the latter for a few days at room temperature, it should be known that chloroform shares this power with a series of other substances, among which may be quoted as instances: toluene, butyl- and amylalcohol, sulfuric ether, a great many esther-salts such as ethyl-butyl- or amylacetate, ethylpropionate, and so on. All of these substances can, in common with chloroform: (1) clot cell-free bird plasma; (2) accelerate the clotting of recalcified oxalate plasma of the dog, the rabbit and of other mammals; (3) enable the clot to fibrinolyze; (4) alter the coagulant property of thrombin in the manner described above as regards chloroform.

I consider that the change undergone by thrombin during the digestion of the serum saturated with chloroform, is a phenomenon akin to fibrinolysis. I regard the fibrin as the product of the union of a proteolytic enzyme, thrombozym, with two proteins of the plasma: thrombogen and fibrinogen. Thrombin is the product of the union of thrombozym with thrombogen alone.¹ Fibrin and thrombin are, in this sense, both the products of the clotting of the plasma. If the plasma of mammals contained a quantity of fibrinogen equal, in terms of equivalence, to its content in thrombozym and thrombogen, the only product of the clotting would be fibrin. The word "thrombin" does not indicate a fixed chemical entity. It applies to the whole series of aggregates not saturated with fibrinogen, which arise from the clotting of the plasma, but, unlike fibrin, remain in solution. All these aggregates have a nucleus made up of thrombozym and thrombogen. Because of their affinity for fibrinogen, they unite with it until it is wholly consumed. They also have an affinity for other proteins than fibrinogen, proteins which are also produced by the liver, such as antithrombosin, but they are more soluble. Once the fibrinogen is used up, the excess aggregates of thrombozym and thrombogen gradually satisfy their remaining free affinities by combining with these more soluble hepatic proteins or also, perhaps, by joining with one another. This phase of clotting is much slower than the formation of fibrin. It occurs during the following hours, and leads to the loss of the coagulant property of the thrombin, to its change into metathrombin.

¹ A Schmidt's prothrombin, as it is present in the plasma, is not a single substance, but a mixture of thrombozym and thrombogen in a free state.

Under the promoting influence of chloroform and similar agents, all these soluble aggregates are proteolysed by the thrombozym they contain, just as is fibrin; thrombogen, their antiproteolysing component, is gradually destroyed, in such a manner that these aggregates tend to become a substance which, if it is not the original thrombozym, is at least very much akin to it and may be called the thrombin of autolysed serum. That is why thrombin ceases to be coagulant for pure fibrinogen but retains its proteolysing property. This conception gives a simple explanation of the fact that all the substances that promote the clotting of a plasma and the formation of thrombin, also promote fibrinolysis and the transformation of the coagulant properties of thrombin. For if coagulation is a preparatory step to proteolysis, it can be readily understood that the same agent should promote both.

FLUIDITY OF THE BLOOD IN THE VESSELS

It is a fact that the plasma contains all the elements required for the clotting of blood and for the formation of a considerable amount of thrombin. How, therefore, shall the blood remain fluid in the vessels, and even, where the blood of oviparous vertebrates is concerned, in contact with bare glass? Surely not for want of an essential factor, as A. Schmidt and his partisans would have it. If the authors who confined themselves to the study of the blood of mammals, had, like Wooldridge, devoted their attention to the plasma of the dog injected with peptone, they would not have been astonished by this fact. Peptone plasma, even more than bird or fish plasma, is stable, that is to say, it remains fluid indefinitely in contact with glass.

Not only does it not clot, but it also prevents the clotting of many times its own volume of normal blood with which it is mixed. And yet, as Wooldridge discovered sixty years ago, it is not because it fails to possess the elements required for the formation of fibrin and thrombin. After dilution with distilled water and bubbling of CO_2 through it, clotting occurs and a large quantity of thrombin is found in the serum.

The stability of peptone plasma is due to the lavish presence in this liquid of the anticoagulant principle called antithrombosin, which is produced in the liver. Antithrombosin exists not only in the plasma

of a dog injected with peptone, but also in normal dog plasma. To its presence in the plasma of the normal animal must be attributed the fact that oxalate plasma at (0.15 per cent) withstands the anticoagulant action of a quantity of thrombin many times greater than that required to coagulate the same volume of the oxalated solution of pure fibrinogen issued from this plasma (P. Nolf, 1919) (21). It flows into the blood from the liver partly directly, and partly through the lymphatic vessels of the liver and the thoracic duct (Shore, 1890). The examination of the thoracic lymph shows that the liver secretes it, in quantities all the greater as the tendency to coagulation is more marked. The antithrombotic function of the liver plays a part in the protection against the danger of intravascular clotting (P. Nolf, 1910) (22).

Having measured the quantities of antithrombosin which are contained in a number of different kinds of plasma, all of them cell-free, before and after their coagulation, I find that the clotting is always accompanied by:

- (1) The production of a large excess of thrombin, as Wooldridge had shown;
- (2) A more or less complete consumption of antithrombosin.

The two phenomena are linked, as cause to effect, in such a manner that the greater the production of thrombin, the smaller the remaining quantity of antithrombosin. This latter substance is veritably consumed during the coagulation. In all probability one part is fixed in fibrin, the other combines with thrombin so as to produce meta-thrombin.

Wooldridge made in 1888 an experiment, the interest of which was not sufficiently appreciated (23). He showed that an emulsion of lymphocytes in isotonic saline exercises on peptone plasma a coagulant action which, if it is weaker than that exercised by the same bulk of an aqueous extract of lymph glands, is however very marked. Using two peptone plasmas, the one very anticoagulant, which he called the strong plasma, the other not so anticoagulant, which he called weak, he determined, in a preliminary experiment, what quantity of lymphocytes emulsion was just needed to coagulate a given volume of weak plasma. This quantity added to a similar volume of strong plasma, left it fluid. Having then separated the lymphocytes from the super-

natant strong plasma he carried them over into the weak plasma. The latter was not coagulated, so that the lymphocytes having been immersed in the strong plasma, had lost their coagulant action on the weak plasma. Wooldridge's experiment does not require two distinct plasmas; it can be made with one plasma, half of which is first heated at 56°C . so as to deprive it of its fibrinogen and yet preserve its anti-coagulant power. A given quantity of the lymphocytes, which would coagulate the fresh plasma on being added to it, lose their coagulant influence if they are previously immersed in the heated plasma. But this loss is not the only alteration they undergo. Before this treatment, the emulsion of lymphocytes exercised no appreciably coagulant action on a solution of fibrinogen provided with the required amount of calcium salts. After being soaked in heated peptone plasma, the lymphocytes acquire the property of coagulating fibrinogen, so that heated plasma makes them clot fibrinogen and at the same time deprives them of their coagulant influence on fresh peptone plasma (P. Nolf, 1908) (24).

These facts can be explained as follows: After they have been washed, the lymphocytes still contain a certain amount of thrombozym or a substance akin to it adhering to their protoplasm. When added to peptone plasma previously heated at 56°C ., they fix thrombogen on their surface so as to be covered with a film of thrombin. The latter is capable of uniting either with fibrinogen to form fibrin, or with antithrombosin, if the latter is in excess in the medium, to form metathrombin, so that when the lymphocytes, after a few minutes' contact with the heated plasma, are carried over into pure fibrinogen, they coagulate the latter, whereas when added to peptone plasma, they find in it such an excess of antithrombosin that they are unable to coagulate the fibrinogen. Wooldridge's experiment suggests a plausible explanation of the fact that blood can remain fluid in contact with the endothelium of vessels; and lymph, with all of the cells of the tissues. Like the lymphocytes in Wooldridge's experiment, endothelium and tissue cells are incapable of clotting blood or lymph granted that these liquids carry a sufficient quantity of antithrombosin. Antithrombosin therefore, seems to be the main agent of the stabilisation of these colloïdal media that are called plasma or lymph. It rests with the liver to furnish these liquids with a sufficient quantity

of antithrombosin to ensure stability. This organ reacts by producing an increased quantity of antithrombosin whenever the tendency of the blood to coagulate is increased (22).

THROMBOPLASTIC ACTION

The influence of the anticoagulant factor having been described, it remains to examine how those substances which promote clotting such as chloroform and so on, work. According to the current theory, all clotting ultimately amounts to the formation of thrombin at the expense of the prothrombin of the plasma, followed by the action of this thrombin on the fibrinogen. The first of these phenomena is supposedly due to the liberation by the platelets of a substance which works on the prothrombin of the plasma. This substance is called thrombokinase by those who consider it to be of an enzymatic nature (P. Morawitz), and cytozym by those who hold it to be a phosphorylated lipid (J. Bordet). In reality, therefore, for all those who uphold A. Schmidt's theory, the greater or lesser speed at which blood clots, depends on the quantity of the substance released by the platelets. In this, as in many other points, the current theory falls short of the actual facts, since it fails to explain the indisputable coagulant action on cell free plasma of a great number of agents which have nothing to do either with thrombokinase or with cytozym. These range from the contact of plasma with glass to the strong coagulant action of chloroform. That contact of the blood with wetted surfaces materially hastens coagulation, has been known for a long time. The cause for this phenomenon is usually given as being an irritating action on the white cells and platelets followed by a liberation of the active substances contained in these elements. In reality, contact with a glass surface can produce the clotting of an entirely cell-free plasma. The clotting is accelerated in proportion as the contact between plasma and glass is extended. Thus, for instance, fish plasma, which remains indefinitely fluid when kept at ordinary temperature in a glass tube, clots after a few minutes, if glass is added to it, after having been ground to a very fine powder in an agate mortar (25). On the other hand, if in a glass vessel containing fish plasma, a few very thin glass tubes are introduced, the plasma will enter the tubes by capillarity and clot inside them, whereas outside of them it remains fluid (26).

Another interesting example of the influence of surface is given by the action, at 37°C., of calcium oxalate on peptone plasma (18). To 2 cc. of plasma, which must not be too strong (not too anticoagulant), 0.05 cc. of sodium oxalate at 3 per cent is added first, and then 0.05 cc. of calcium chloride at 11.1 per cent. The calcium oxalate does not precipitate immediately. The plasma merely becomes very opalescent and shows a reddish color against the light. The calcium oxalate is, at the moment, in colloidal suspension in the plasma. After a few minutes, the oxalate precipitate appears, first as very fine flocculi which, as they cover themselves with a thin layer of fibrin, agglutinate, whereas the whole mass of the liquid sets to a gel. In a control, 0.05 cc. of the oxalate solution is mixed with 0.05 cc. of the calcium solution and left to stand for a while. Only after the precipitate of calcium oxalate has completely settled and taken a crystalline form, 2 cc. of the same peptone plasma are added to the precipitate and both are gently shaken together. The plasma remains indefinitely fluid. There is but one difference between the two experiments: the degree of dispersion of the calcium oxalate. In the first, the dispersion is great, the calcium oxalate being in a colloidal state; under these conditions, it is highly coagulant for peptone plasma. In the second, the dispersion is far less, the substance being crystallized, and its coagulant power falls to nothing. Clotting of peptone plasma by nascent calcium oxalate leaves, as after any clotting of plasma, a serum containing thrombin and entirely free of any antithrombin.

With the coagulant action of ground glass, or of calcium oxalate in colloidal suspension, should be connected also that of chloroform, discovered by Howell, and of other liquids which are not very soluble in plasma and liable to emulsify in the latter. Chloroform exercises a coagulant action, when it is simply dissolved in the plasma; but its coagulant power increases, if it is in excess in the liquid as a fine emulsion. Under these conditions, as has been said above, chloroform clots in a few minutes a bird plasma which, without the admixture of chloroform, would have remained indefinitely fluid. Here again, as in the case of the clotting of fish plasma by ground glass, or that of the clotting of peptone plasma by nascent calcium oxalate, coagulation visibly begins by the agglutination of the suspended particles. These are already rendered viscous by a deposit of fibrin and agglomerate

at a moment when the plasma is still fluid. It is only after a certain time that the plasma itself sets into a firm gel.

This separation of coagulation into two successive phases: agglutination of suspended particles and gelification of plasma, is an interesting fact, because an analogous phenomenon occurs in normal blood clotting which concerns the platelets and has been termed agglutination, or viscous transformation of the platelets. The partisans of A. Schmidt's theory have always considered it a self-evident fact that these changes in the platelets are the preliminary condition of the gelification of the plasma. On the contrary, it seems far more likely that agglutination of platelets and gelification of plasma are two consecutive steps of one phenomenon, which is the formation of fibrin from its constituents present in the plasma. A current manner of stating the relations between agglutination of platelets and coagulation of blood is to say that all of the agents which prevent the changing of the platelets, also impede the coagulation of blood. It is more in agreement with facts to hold that all of the means which prevent the coagulation of the cell-free plasma, also prevent the alteration of the platelets in the shed blood.

Under the influence of ground glass, of colloidal calcium oxalate, or of emulsified chloroform, a plasma which, left to itself, would have remained fluid indefinitely, clots, and in the serum appears a more or less considerable quantity of thrombin, whereas thrombozym, thrombogen and antithrombosin disappear. The existence of these facts is not to be denied. A. Schmidt's theory cannot account for them. It is silent about those many substances, insoluble or soluble, which are entirely foreign both to the media of the living organism and to the living cells, and which are nevertheless able to provoke the full coagulation of a given plasma and to produce in the serum a great excess of thrombin. This coagulant action occurs in a totally cell-free plasma, it has nothing to do with any interference of white cells or of platelets. To designate it, I have introduced the term: thromboplastic. The thromboplastic agent has no part in the constitution either of thrombin or of fibrin. These are formed exclusively from their precursors contained in the plasma. The thromboplastic agent intervenes solely to make the reaction possible between these precursors. It acts as a catalysator. And so also do the alcoholic or aqueous tissue extracts.

THROMBOPLASTIC ACTION OF FIBRIN AND THROMBIN

This leads us to consider in their turn the action of fibrin and of thrombin on the clotting of a stable plasma. The first, when finely divided, as well as the second, which is soluble, has a coagulant action on plasma. For those who stand by A. Schmidt's interpretation of coagulation, the clotting of a stable plasma by thrombin cannot be conceived of otherwise than as the result of the action of this thrombin on the fibrinogen of the plasma. The objection can be raised against this explanation that it does not apply to fibrin, the coagulant action of which is nevertheless undisputable. In actual fact, fibrin acts on a natural plasma just like thrombin, and not in the manner described above. The following experiment may be quoted in support of this statement. Blood of mammals is received in its volume of 10 per cent sodium chloride. The cell-free plasma separated from this mixture (salt plasma) clots after dilution with four volumes of water. The coagulation takes a certain time to occur. The fresh serum contains, as in any clotting of plasma, an appreciable quantity of thrombin (Wooldridge). Added to the salt plasma after the latter has just been diluted with four volumes of water, it accelerates to a notable degree the clotting of the plasma. It also coagulates oxalate plasma (0.1 per cent of oxalate). If the serum is left to stand during one or two days, it loses completely its coagulant action on the oxalate plasma, while keeping entire its coagulant action on the salt plasma. Thus the mode of action on the salt plasma differs from a simple transformation of fibrinogen into fibrin by the thrombin contained in the serum. Bordet and Gengou think that in the stored serum thrombin has altered, that it has lost its property to make fibrinogen insoluble, but has kept that of transforming the prothrombin of the diluted salt plasma into thrombin (27). It is simpler to admit that the mode of action of the stored serum is of the same nature as that of all the substances which promote the coagulation of a normal plasma. In other terms, it is thromboplastic (P. Nolf). In this explanation, the new formation of thrombin in the salt plasma under the influence of the stored serum, is only one instance of the general rule established by Wooldridge, that every coagulation of a normal cell-free plasma is always accompanied by the formation of thrombin.

The mode of action of the thromboplastic agent is to destroy the

colloidal equilibrium of the plasma by promoting the interaction between thrombozym and thrombogen so as to facilitate the production of thrombin and fibrin; and the latter, as they appear, add their own thromboplastic influence to the already existing ones. It is, therefore, not correct to assert, as does H. Eagle, that the fact that I consider thrombin as a product of clotting amounts to denying the latter any importance in the evolution of the phenomenon. As soon as, under the influence of a thromboplastic agent, the clotting of a plasma is set going, the process seems to provide itself with an increasing impetus for its further development, by producing new thromboplastic agents, thrombin and fibrin, and consuming the inhibiting factor, antithrombosin. Plasma clotting, therefore, seems doubly like an autocatalysis, as I brought out explicitly in 1912 (28).

All that has been said of the plasma is even truer of the whole blood containing its cellular elements. Everybody admits that coagulation begins by an agglutination of platelets followed by their disintegration. The phenomenon occurs when the greater mass of the plasma is still completely fluid, which does not prove that it precedes any changing of the plasma. In reality, the agglutination and disintegration of the platelets are the first token of the clotting of the plasma, just as the agglutination of the particles of calcium oxalate anticipates the coagulation of the peptone plasma. The first deposits of fibrin occurring on the surface of the platelets cause the viscous metamorphosis of the latter and the liberation of the coagulant factors they contain. Here again, even more so than for cell-free plasma, clotting as soon as it begins, endeavours to create for itself conditions more and more favourable to its own development.

Clotting is like the fall of an avalanche or the deflagration of gun powder. This character is clear in the light of my theory, but hardly fits in with an explanation of clotting according to which the latter is a succession of two phenomena: the transformation of prothrombin into thrombin and the action of thrombin on fibrinogen (28).

THE COAGULATION OF OXALATE PLASMA WITHOUT ADDITION OF CALCIUM SALTS

One last discrepancy between the usual theory of blood clotting and facts, concerns the undeniable coagulant action of chloroform on

oxalate plasma, discovered by Howell. It is to be seen in birds as well as in mammals. It belongs also to many of the substances which can be used as substitutes for chloroform, such as amylalcohol ethyl-, propyl-, butylacetate, ethylpropionate, butyrate, and so on. The clotting of oxalate plasma by chloroform is slower than that of the plasma provided with calcium salts; and the serum left after the clotting is far less rich in thrombin. But, save for this difference in degree, the two phenomena are similar. One readily understands that the partisans of the usual theory should consider this clotting of oxalate plasma by chloroform an obnoxious fact, since the whole theory is based on the supposedly different nature of the formation of thrombin out of prothrombin, and of the action of thrombin on fibrinogen, the first of these phenomena demanding the presence of calcium, whereas the second does not need calcium. The partisans of the said theory have, accordingly, either been silent about the fact or else have evaded the issue, by asserting that the thrombin found in the oxalate plasma after its clotting by chloroform preëxists, but is prevented by the presence of antithrombosin from clotting the fibrinogen. According to them, the part of chloroform is to destroy the antithrombosin, and to make clotting possible. No experimental datum has been alleged to support this opinion which is, furthermore, contradicted by certain results obtained by me (12). I added chloroform to a series of plasmas first freed of the greater part of the precursors of thrombin by various treatments, (adsorption by tricalcium phosphate, heating at 56°C., elimination of euglobulin). I found that after these liquids have been subjected to the action of chloroform there is but little or no thrombin to be encountered in them. If the antithrombosin is measured before and after the action of chloroform, it is noted that antithrombosin only disappears, on condition that thrombin has been produced. In the absence of thrombin, chloroform is completely devoid of any destructive action on antithrombosin. Here as usual, the consumption of antithrombosin does not precede the production of thrombin, but is a consequence of this production. Contrary to the explanation imagined by the upholders of the classical theory, the clotting of oxalate plasma by chloroform differs in no essential trait from the clotting of the same plasma provided with calcium salts. On both sides, thrombin and fibrin are produced. On both sides, also,

fibrin and thrombin subsequently undergo the same process of proteolysis. There is no other difference between the two but this: in the presence of calcium salts, clotting is more massive and ultimately leads to the formation of greater quantities of thrombin and to a more thorough consumption of antithrombosin.

The coagulant action of chloroform on oxalate plasma is an interesting fact, which it would be unwise to pass over, for the substances are becoming more and more numerous that are known to clot oxalate or citrate plasma and have no influence on a fibrinogen solution. The following may be quoted as examples: certain snake venoms, microbe toxins (staphylotoxin) autolysed chloroform serum from the dog or the fowl, trypsin, and so on. Many authors explain these clottings in a manner one might call orthodox. Considering it an axiomatic truth that, in the absence of calcium salts, fibrinogen can be clotted only by thrombin, they take it that the above-named agents are capable of playing the part of thrombin. If we held this explanation to be valid in all cases, we should have to class chloroform amongst the substitutes for thrombin, which is obviously untrue. Shaken with chloroform, a solution of fibrinogen, granted it is pure, is rendered partially insoluble by it, but not clotted. The precipitated fibrinogen redissolves when the chloroform is evaporated, and can then still be clotted by thrombin (P. Nolf (29)).

In fact, the current theory of blood coagulation fails to explain why the above-named agents clot oxalate or citrate plasma and yet do not clot fibrinogen. These substances, instead of acting like thrombin, act in a diametrically different manner; since, as recalled in this article, thrombin clots a solution of fibrinogen far more easily than the same volume of oxalate plasma.

CONCLUSION

If A. Schmidt's theory explained the innumerable facts brought up by experimentation, there would be no reason why it should not be adopted by all the authors who have studied the problem of blood coagulation. New explanations of these phenomena are set forth everyday not, as H. Eagle says in his article: "Recent Advances in the Blood Coagulation Problem," "because the experimental data on which they are based are either in error or incomplete," but more

probably because many authors are unable to make the facts they have observed, fit in with A. Schmidt's theory. As for the partisans of the latter, they seem to have adopted the easy method of simply ignoring those facts which they find too unruly.

As a result, there is no chapter of physiology about which ideas are as confused as about blood clotting. Despite the great number of works published every year on it, opinions are diverging more now than twenty-five years ago. To my mind, and here I am glad to agree with H. Eagle, no further progress can be made but by a return to the scientific method. Setting aside any and all preconceived ideas, we should endeavour to test the accuracy of the experimental methods and the validity of the findings. It would be good, amongst other things, if A. Schmidt's upholders took the trouble to check those results which are known not to tally with their theory. This article was written mainly to invite them to do so.

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PLASMA FIBRINOGEN RESPONSE IN MAN

INFLUENCE OF THE NUTRITIONAL STATE, INDUCED HYPERPYREXIA, INFECTIOUS DISEASE AND LIVER DAMAGE

THOMAS HALE HAM, M D AND FANNY C CURTIS, A B

*From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard
Boston City Hospital, and the Department of Medicine,
Harvard Medical School, Boston*

I	Introduction	41
II	Methods	41
III	Level in Normal Human Subjects	41
IV	Influence of Diet	41
	A Nutritional Deficiency	41
	B Protein Ingestion in Normal Subjects	41
	C Protein Ingestion in Subjects with Previous Deficiency of Dietary Protein	42
	D Summary	42
V	Relation of Fibrinogen Response to Fever	42
	A Intravenous Injection of Typhoid Vaccine	42
	B High Environmental Temperature	42
	C Summary	42
VI	Fibrinogen Response to Infection	42
VII	Failure of Fibrinogen Response	43
VIII	Discussion	43
IX	Conclusions	43
X	Bibliography	44

I INTRODUCTION

An elevation in the concentration of plasma fibrinogen is one of the most frequent responses of the organism to a variety of noxious stimuli (18, 19, 28). Fibrinogen production, as indicated by an increase in the plasma level, may be accelerated by tissue inflammation or destruction, whether traumatic, chemical, bacterial or neoplastic in origin (15, 18, 19, 25, 39, 54, 60, 74, 80). It may be increased following the injection of foreign protein, proteose or peptone, small doses of liver poisons, and by damage from Roentgen-rays (19, 28, 70). Fibrinogen production is increased in pregnancy (7, 8, 62) and by a diet rich in animal protein, but is decreased by starvation (17, 76).

and may be decreased or completely inhibited by severe liver damage or impairment of liver function (9, 19, 24, 41, 49, 51, 52, 53, 67, 70, 80, 81).

Certain aspects of the normal and pathological levels of plasma fibrinogen have been defined from the direct chemical determination of the fibrin content of the plasma of human subjects and experimental animals by Gram (26, 28), Foster and Whipple (15, 17, 18, 19), Fahraeus (12, 13), Starlinger and coworkers (43, 74) and others (11, 21, 32, 65). Further knowledge of plasma fibrinogen variations in health and disease has been obtained indirectly from the numerous observations of the erythrocyte sedimentation rate, which frequently serves as a rough index of the fibrinogen concentration (1, 3, 11, 12, 22, 23, 30, 36, 64, 78, 79, 82). The sedimentation rate, however, occasionally is accelerated in instances of abnormal concentrations of serum globulins and, rarely, in lipemia (3, 12, 34, 35, 40, 44, 45, 75). The sedimentation rate of erythrocytes is discussed separately in the subsequent paper in this Journal.

The purpose of the present communication is to determine the effect of nutritional deficiency and of protein feeding on the fibrinogen level in man; to compare the fibrinogen response to fever induced by two artificial methods with the responses observed in infectious disease; and, finally, to present data concerning 4 patients with varying degrees of liver damage who showed a failure of fibrinogen response. The plasma fibrinogen response is compared to the variation in serum proteins, leukocytes and body temperature.

II. METHODS

The proteins were determined on samples of plasma, using dry potassium oxalate as anticoagulant, in a concentration of 500 mg. per 100 cc. of whole blood. The plasma fibrinogen was removed as fibrin by the clotting method of Cullen and Van Slyke (6). In instances of low or subnormal values for fibrinogen, purified cephalin (48) was added to one of the duplicates or to the fibrin-free filtrate, which was allowed to stand and was examined for further fibrin formation. The albumin-globulin separation was performed by Howe's method (37, 38), employing 22.5 per cent sodium sulfate. For determination of the non-protein nitrogen the proteins were precipitated by the

tungstic acid method of Folin (14). All nitrogen determinations were made by the micro-Kjeldahl method. Acid digestion was performed employing the Arnold-Gunning mixture (61) and potassium persulfate as the extra-oxidizing agent (84). The acid digest was distilled by the Bock-Benedict technique (4), followed by nesslerization of the distillate. All protein determinations were done in duplicate and the results of the micro method were checked satisfactorily in several instances by the macro-Kjeldahl procedure. The sedimentation rate was observed by the method of Rourke and Ernstene (69), reported as the corrected sedimentation index, in millimeters per minute.

III. LEVEL IN NORMAL HUMAN SUBJECTS

Although the fibrinogen concentration of normal human subjects has been investigated frequently (15, 23, 28, 31, 33, 52, 74), many determinations are not acceptable, either because of the method of fibrin analysis or because of failure to establish the status of the subject's health. The fibrin method of Foster and Whipple (16) may be unreliable for low concentrations, as demonstrated by Jones and Smith (41). The frequency of mild infectious diseases causing significant elevation of fibrinogen requires the critical selection of "normal" subjects. The data of three series from the literature (23, 28, 29, 74) together with the data reported in this communication are summarized in Table 1 and indicate that the usual limits of the fibrinogen concentration for normal adult subjects are approximately 190 mg. to 330 mg. per 100 cc. of plasma, with the more usual range from 220 mg. to 280 mg., and with an average concentration of 250 mg. In these data there is no outstanding variation in the fibrinogen concentrations for various age groups or for males and females.

Gram (28) and Rourke and Plass (68) have shown that the concentration of fibrinogen does not vary significantly following the ingestion of food or after moderate exercise. The fibrinogen level has been shown by Gram (28) and by others (52, 74) to be relatively constant during health in the same individual over a long period of time. Likewise, in the observations reported below, food ingestion and short violent exercise had no significant influence on the fibrinogen concentration. The range of variation of 28 determinations of plasma fibrinogen observed during a period of 16 months in a normal 31-year-

old male was from 220 mg. to 260 mg., with a maximum variation of 18 per cent. In 3 other normal male subjects of 61, 34 and 37 years, observed during periods of 3 months, 10 days and 7 days, respectively, the maximum variation in fibrinogen concentration for each subject was 18, 16 and 14 per cent with respective ranges in concentration of from 250 mg. to 300 mg. for 9 determinations, from 240 mg. to 280

TABLE 1
Plasma fibrinogen level in normal human subjects

AUTHOR	METHOD	NUMBER OF DETERMINATIONS	NUMBER OF SUBJECTS	AGES	PLASMA FIBRINOGEN CONCENTRATION	
					Range	Average
Gram (28)	Gravimetric, Gram (26)	25	25 Males	16-19	200-360*	270
		25	25 Females	20-52	210-380*	290
Starlinger and Winands (74)	Gravimetric, Starlinger and Hartl (73)	25	1 Male 15 Females	20-32	220-360*	250
Gilligan and Ernstene (23)	Colorimetric, Wu (85)	43	25 Males 12 Females	15-35	200-310	250
Present communication	See Methods	54	19 Males	25-61	190-330	250
		21	9 Females	20-35	220-290	250
Total		193	70 Males 61 Females	16-61	190-380*	250 (approximate)

* Fibrinogen levels of 360 mg. or above occurred 3 times in Gram's series, once in the series by Starlinger and Winands.

mg. for 10 determinations, and from 220 mg. to 250 mg. for 7 determinations.

IV. INFLUENCE OF DIET

The influence of diet on the fibrinogen level has been observed in dogs by Foster and Whipple (17) and by Vars (76). For human subjects Starlinger and Winands (74) have reported low normal fibrinogen levels in nutritional deficiency diseases, including pernicious

anemia. In the observations described below the fibrinogen concentration was determined in human subjects who had received diets deficient in animal protein or vitamins, or in both. The subsequent influence of diets rich in protein was studied in certain of these patients and in normal subjects.

A. Nutritional Deficiency. In 16 patients with nutritional deficiency, including 12 cases of pernicious anemia, 3 cases of scurvy and 1 case of pellagra, a history of inadequate ingestion of protein was obtained and the lack of adequate vitamin ingestion inferred in the cases of scurvy and pellagra. In the patients with uncomplicated

TABLE 2

Plasma fibrinogen level in uncomplicated pernicious anemia before remission, on a diet containing no meat or eggs

CASE	SEX	AGE	PLASMA FIBRINOGEN	R B C.	HEMOGLOBIN
			mg per 100 cc.	millions per cu mm	per cent
1	F	73	170	0.74	20
2	M	63	180	1.23	50
3	F	43	190	1.82	46
4	M	64	230	1.11	27
5	M	74	240	3.33	70
6	M	40	240	0.89	26
7	F	55	250	1.84	49
8	F	64	270	1.83	49
Average ..			220		

pernicious anemia, Cases 1 through 8 in Table 2, the fibrinogen level determined before treatment, while the patients were maintained on a diet containing no meat or eggs, varied from 170 mg. to 270 mg. per 100 cc. In 1 patient with uncomplicated scurvy, Case 13, the fibrinogen concentration was 250 mg.

Two patients with pernicious anemia, Cases 9 and 10, were maintained on diets deficient in protein during active remissions from the anemia induced by liver extract. As shown in Table 3, the plasma fibrinogen level in Case 9 after $8\frac{1}{2}$ weeks was below the normal level. Case 10 received the "basal" diet, consisting of the following foods only; potato, rice, macaroni, white bread, orange juice, ice cream,

tea, coffee and sugar. In Case 10, as shown in Table 4, the fibrinogen concentration during a period of 6 weeks decreased from normal to an abnormally low level of 160 mg., with a corresponding decrease of total plasma proteins from 6.2 to 5.2 grams per cent and a decrease of serum albumin from 4.0 to 3.6 grams per cent.

TABLE 3

Plasma fibrinogen level in Case 9, a male of 52 with uncomplicated pernicious anemia during remission

PLASMA FIBRINOGEN	R.B.C.	HEMOGLOBIN	WEEKS	TREATMENT
mg. per 100 cc.	millions per cu.mm.	per cent		
—	1.65	39	1	Diet of 46 grams protein; no meat or eggs
—	2.67	68	7	Daily intramuscular injections liver extract for 7 weeks; diet same
190	2.59	73	8	Diet same; no injections of liver extract
190				
170	2.97	75		
170				
180	3.45	76	9	
180				
180*	3.22	79	1	The above diet with the addition of 93 grams of protein daily derived from 454 grams of raw liver pulp
220*	3.39	86	2	
240*	4.16	89	3	
240*	4.29	93	4	
240*	4.27	89	5	
—	4.80	97	6	
270*	4.87	100	7	
290*	5.09	101	8	
270*	5.26	97	9	
270*	5.59	111	10	

* Average concentration (3 to 7 determinations per week).

In 6 patients with nutritional deficiency the fibrinogen concentrations were increased above normal, however, in response to complicating infections. Thus in Case 10, Table 4, during the subsequent 5 weeks on the same low protein diet the fibrinogen increased to the abnormal value of 410 mg., associated with phlebitis. The low concentrations of total protein and albumin, however, were maintained.

In 2 additional patients with pernicious anemia, fibrinogen values above normal were observed following an uncomplicated bone marrow biopsy in Case 11 and associated with lobar pneumonia in Case 12, the fibrinogen concentrations being 350 mg. and 960 mg., respectively. In 2 patients with scurvy, Cases 14 and 15, and in 1 patient with pellagra, Case 16, all complicated by respiratory infections, the plasma fibrinogen concentrations were, respectively, 420 mg., 410 mg. and 480 mg.

B. *Protein Ingestion in Normal Subjects.* Foster and Whipple (17) in observations on dogs found that diets rich in various animal proteins

TABLE 4

The influence of low protein diet on the plasma fibrinogen level in Case 10, a female of 63 with pernicious anemia, observed before and after an infectious complication

PLASMA FIBRINOGEN	R.B.C.	HEMOGLOBIN	DAYS	TREATMENT AND COMPLICATIONS
mg. per 100 cc.	millions per cu.mm.	per cent		
230	1.10	25	2	No complication. Treatment: liver extract by mouth; diet low in protein, no meat or eggs
170	1.22	25	24	
160	1.23	29	28	
160	1.14	29	35	
160	1.33	32	42	
230	1.45	35	49	Phlebitis of both thighs with suppuration of left thigh. Treatment and diet as above
310	2.13	48	56	
330	2.43	53	63	
330	2.49	53	69	
410	2.90	55	77	

avored a high fibrinogen level, in contrast to fasting or diets rich in carbohydrate or fat. In some instances a diet of cooked pig's stomach produced an increase of plasma fibrinogen of 100 per cent above the fasting level in from 3 to 4 days. In one experiment these investigators reported a fibrinogen increase of 40 per cent on the same day in which a 30-pound dog, previously fasting, was fed 4 pounds of cooked pig stomach. Vars (76) in similar experiments observed no differences in the response to proteins from various animal sources, i.e., pig stomach, liver, kidney, casein and "meat residue." Starvation periods of 3 to 6 days usually produced minimum values for fibrinogen.

In the observations reported below the influence of protein feeding on the fibrinogen level was investigated in 2 normal men. One received relatively large amounts of cooked pig stomach; the other received raw liver pulp and, subsequently, cooked pig stomach. It must be recognized, however, that it is impossible for human subjects to ingest amounts of these substances comparable to those eaten by a dog (17); i.e., a 150-pound man would be required to ingest 20 pounds of cooked pig stomach in one day to be comparable to a 30-pound dog eating 4 pounds. Fresh pig stomachs, trimmed free of fat and connective tissue, were boiled for 30 minutes and subsequently ground. Excess water was pressed out; the puréed material was

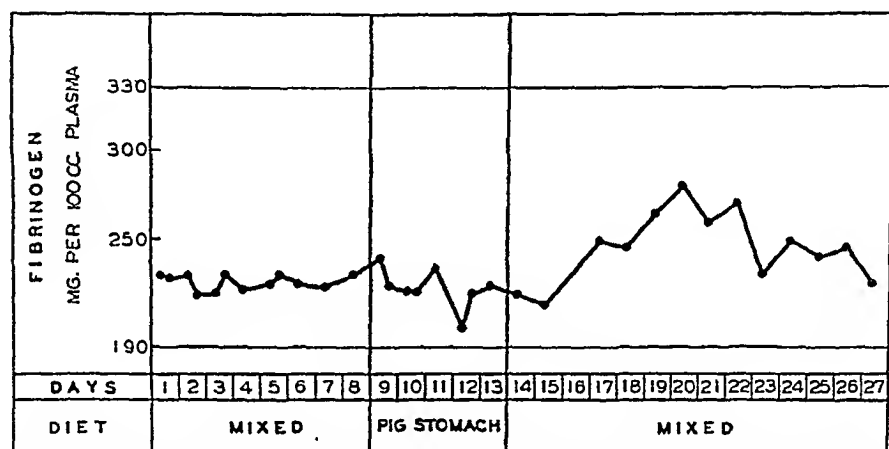


FIG. 1. Plasma fibrinogen response in a normal male of 31 on a liberal mixed diet who received 910 grams of cooked pig stomach (110 grams of protein) per day for 5 days.

weighed and stored in the ice box before being served as meat cakes or mixed in tomato juice. Beef liver was puréed to remove fibrous tissue, the material was weighed and fed in tomato juice.

The first subject, a male of 31 years who received a liberal mixed diet in the initial control period; ingested 910 grams of cooked pig stomach daily, 110 grams of protein, during a second period of 5 days; and returned to the control diet during the third period of observation. There was no significant change in the fibrinogen level during the first two periods, as shown in Figure 1. There then occurred a moderate increase of fibrinogen which reached a maximum concentration, 23 per cent above the average control level, on the seventh day of

the third period, representing a maximum variation of 27 per cent above the control level. This was followed by a gradual return to the control level in the succeeding 7 days.

A second normal subject of 56 years received, in an initial period of 5 days, a control diet containing 80 grams of protein, 80 grams of fat and 320 grams of carbohydrate. During a second period of 5 days the subject ingested 250 grams of raw liver pulp daily, thereby replacing 53 grams of protein of the control diet by an equal amount of protein derived from raw liver. During a third period of 21 days the subject again received the control diet. The fibrinogen increased significantly 24 hours after the ingestion of liver had begun and reached

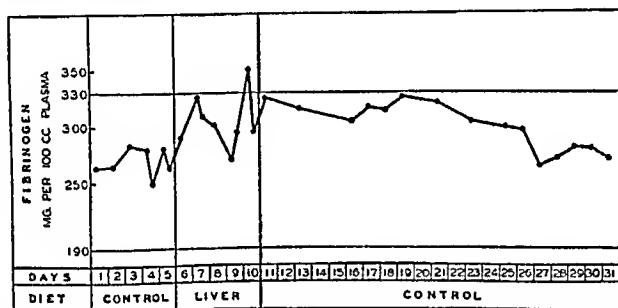


FIG. 2. Plasma fibrinogen response in a normal male of 56 on a control diet containing 80 grams of protein, who received 250 grams of raw liver pulp daily for 5 days, replacing 53 grams of protein in the control diet.

a maximum concentration on the fifth day of the second period, an increase of 31 per cent above the average control level, as shown in Figure 2. Subsequently, in the third period the fibrinogen concentration decreased gradually, but required 16 days to reach the previous level.

In a fourth period of 5 days, immediately after the termination of the above observations, 53 grams of protein of the control diet were replaced by 454 grams of cooked pig stomach daily for 5 days, representing an increase in the total protein intake from 80 grams to approximately 130 grams. During a fifth period of 21 days the subject again received the control diet. The first apparent response occurred

in the fourth period, during the ingestion of pig stomach, when the fibrinogen concentration increased 16 per cent above the average control level on the fourth day of the period. The maximum rise, of 26 per cent, occurred on the second day of the fifth period. The fibrinogen level decreased gradually to the control level during the subsequent 2 weeks of the fifth period.

C. Protein Ingestion in Subjects with Previous Deficiency of Dietary Protein. The influence of protein feeding on the fibrinogen level was investigated in 4 subjects with pernicious anemia in partial remission who had previously received inadequate amounts of dietary protein.

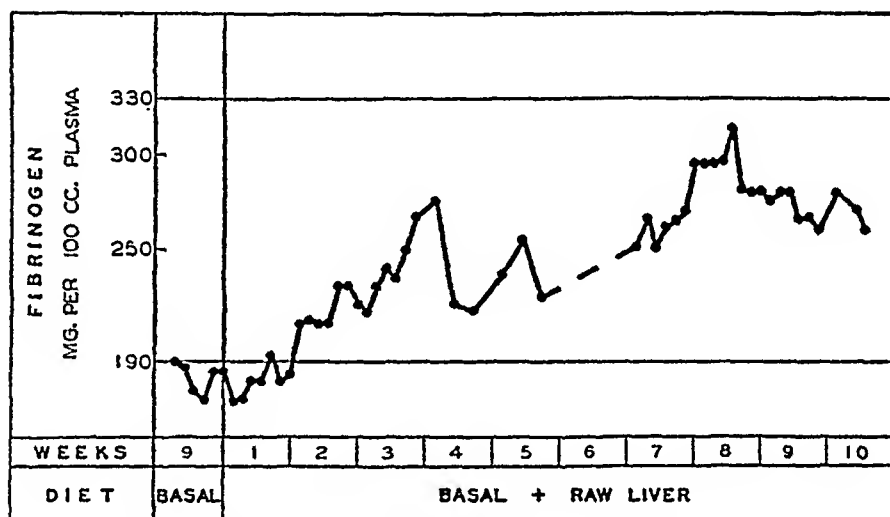


FIG. 3. Plasma fibrinogen response in a patient with pernicious anemia, Case 9, maintained 8½ weeks on a basal diet containing 46 grams of protein (no meat or eggs); subsequently 454 grams of raw liver pulp (93 grams protein) were added to the basal diet daily for a period of 9½ weeks.

One subject, Case 9, discussed above, was maintained initially for a period of 8½ weeks on a diet containing 46 grams of protein and remission was induced by the intramuscular injection of liver extract, as shown in Table 3. The average fibrinogen concentration during the final 6 days of this period was 180 mg., shown as the first period of Figure 3. In a second period of 9½ weeks the patient received the same diet with the daily addition of 93 grams of protein derived from 454 grams of raw liver, a total daily protein intake of approximately 140 grams. Throughout this period the subject remained in negative nitrogen balance, showed a gain in weight of 21 pounds, an increase

in erythrocytes from 2.59 to 5.59 million per cu.mm. and a rise in hemoglobin from 73 to 111 per cent. The fibrinogen level was unchanged during the first week of this regime, but beginning in the second week the level increased gradually and irregularly to the upper limits of normal, as shown in Figure 3. The serum proteins determined throughout both periods were normal and relatively constant in concentration. At no time did the patient present evidence of infection.

As observed above, low fibrinogen values were maintained in Cases 9 and 10, despite remission induced by liver extract, when the diet was deficient in protein. Cases 1, 2 and 3, as shown in Table 2, had abnormally low fibrinogen concentrations before treatment and while on the basal diet. These 3 patients subsequently received the daily addition to the basal diet of a mixture of normal human gastric juice and 50 grams of protein derived from *animal sources*: Case 1 was given 200 grams of boiled pig's stomach mucosa; Cases 2 and 3 each were given 200 grams of beef muscle. In each instance this therapy, maintained for from 13 to 17 days, induced remission from pernicious anemia, as evidenced by a reticulocyte response and an increase of erythrocytes and hemoglobin. In each instance the fibrinogen concentrations had increased significantly and returned to normal values from 3 to 5 weeks after the above treatment. In Case 1 the fibrinogen increased from 170 mg. to 250 mg., in Case 2 from 180 mg. to 250 mg. and in Case 3 from 190 mg. to 270 mg.

D. Summary. The fibrinogen levels of 10 patients with uncomplicated pernicious anemia and 1 patient with uncomplicated scurvy who had taken diets inadequate in protein over long periods were significantly below normal in 4 cases, at the lower range of normal concentrations in 5 cases and approximately at the average normal value in 2. In 2 of these patients, maintained on low protein diets, the fibrinogen level remained at subnormal concentrations or decreased from normal to subnormal concentrations in spite of the remission from anemia induced by liver extract therapy. Nutritional deficiency did not prevent an elevation of fibrinogen above normal in the presence of infection, however, as illustrated in 3 patients with pernicious anemia, in 2 with scurvy and in 1 with pellagra.

In 3 observations on 2 normal human subjects the daily ingestion

for 5 days of from 50 to 100 grams of protein derived from cooked pig stomach or raw liver produced moderate and transient increases in the plasma fibrinogen concentrations. The values exceeded the normal range in one instance. The maximum increases of 25 and 31 per cent above the *average* control level were greater than the maximum variation in normal subjects but were slight compared to the larger increases reported in the observations on dogs by Foster and Whipple and by Vars. This difference probably is due in part to the smaller amounts of protein per kilogram of body weight ingested by the human subjects.

In 1 patient with pernicious anemia the subnormal fibrinogen level, maintained in spite of the remission of anemia induced by liver extract therapy, was increased to the upper limit of normal following the daily addition to the diet of 93 grams of protein derived from raw liver. Similarly, in 3 other patients with pernicious anemia who had received inadequate dietary protein the addition of 50 grams of animal protein to the basal diet produced, during the accompanying remission of the anemia, an increase of fibrinogen levels from below normal to normal concentrations. It is apparent, therefore, that adequate dietary protein, rather than remission from anemia, was responsible for the return of the fibrinogen level to normal in these patients.

V. RELATION OF FIBRINOGEN RESPONSE TO FEVER

It has been established that many *afebrile* conditions, such as chronic low grade infections, pregnancy, traumatic and neoplastic conditions, may be associated with elevated fibrinogen (25, 28, 62, 74). Furthermore, 7 of the 9 patients with uncomplicated pernicious anemia, reported above, showed a significant elevation of body temperature during the week preceding the determination of the fibrinogen, which was normal or below normal in all cases. It is possible, therefore, that fever per se may not be the cause of increased fibrinogen concentrations. This problem was investigated directly in observations on patients who were subjected to one or both of two procedures for the induction of fever: the intravenous injection of typhoid vaccine and high environmental temperature.

Typhoid vaccine was administered intravenously in a single in-

jection, using one of two dosages, either 300 million typhoid bacilli and 225 paratyphoid A and B, or two-thirds of these amounts. High environmental temperature was produced in a simple light cabinet,¹ employing electric light bulbs as the only source of heat. In each observation daily determinations of fibrinogen concentration and leukocyte count were made during an initial control period. In a second period the fibrinogen concentration and leukocyte count were determined at intervals of 2, 5, 12, 24, 36 and 48 hours after the beginning of the febrile period and were determined daily thereafter until the fibrinogen level was constant. The serum proteins were determined on several occasions in each period.

A. Intravenous Injection of Typhoid Vaccine. The intravenous injection of typhoid vaccine was given to 8 subjects, 6 with normal levels of plasma fibrinogen observed during the control period, Cases 17, 18, 9, 19, 20 and 21; and 2 with abnormally elevated concentrations of plasma fibrinogen associated with chronic gonococcal arthritis, Cases 22 and 23.

Of the subjects with normal fibrinogen levels, 3 received the larger dose of vaccine, shown in Figures 4, 5 and 6; the others received the smaller dose. In all 6 subjects the plasma fibrinogen response to the intravenous injection of typhoid vaccine was similar. There was a moderate decrease in fibrinogen concentration 5 hours after injection, no change in 12 hours, and beginning in 24 hours a slow rise to a maximum value which was obtained in from 36 to 72 hours. The subsequent decrease to the previous level was gradual and required from 4 to 10 days. The maximum fibrinogen values observed for the 6 subjects varied from 350 to 420 mg., representing increases above the average control levels of from 23 to 72 per cent; the average maximum increase was 48 per cent for the 6 subjects. The maximum elevation in body temperature produced by the vaccine varied from 101° to 104°F. with a total period of fever above 101°F. of from 1 to 27 hours. The leukocyte response was similar in each case: There was leukopenia 2 hours after injection, maximum elevation above normal in 12 hours and gradual decrease to a normal level within 48

¹ This procedure was carried out at the Massachusetts General Hospital through the courtesy of Dr. Walter Bauer.

hours. The average maximum leukocytosis was 19,200 white cells per cu. mm. for the 6 subjects.

In the 2 subjects with chronic gonococcal arthritis the plasma fibrinogen level was abnormally elevated in the control period in Case 22, as shown in Figure 7; in Case 23 the concentration varied from 530 to 560 mg. Each patient was afebrile before the injection of the larger

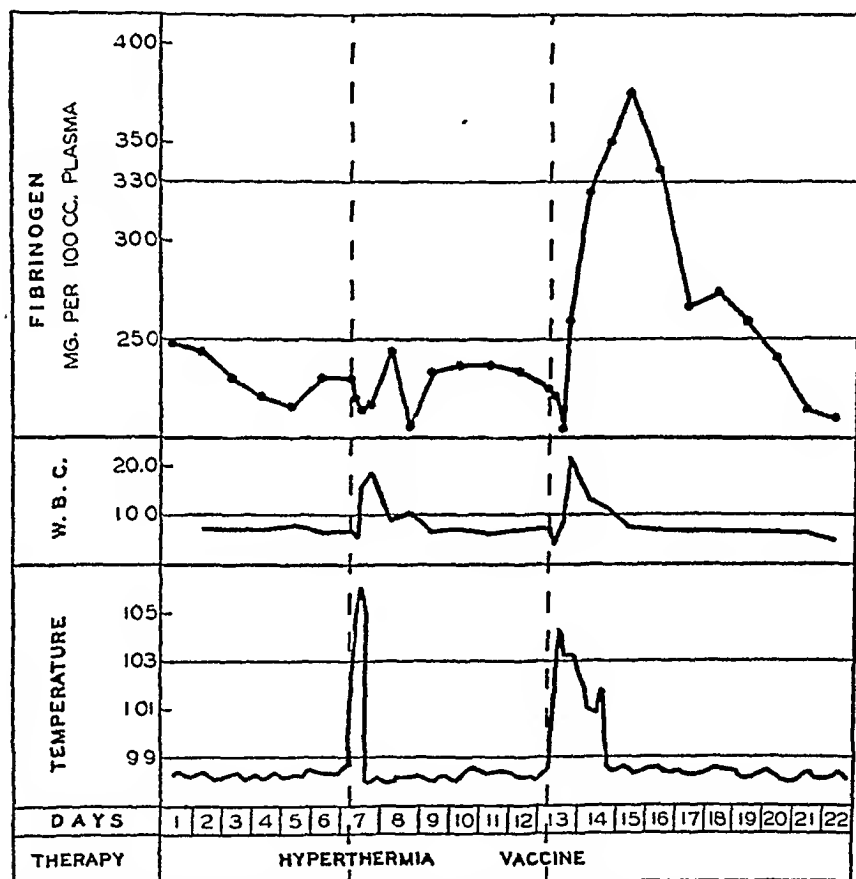


FIG. 4. Plasma fibrinogen, leukocyte and temperature responses in Case 17, subjected to fever as induced, first, by high environmental temperature and, second, by the intravenous injection of typhoid vaccine.

dose of typhoid vaccine. The fibrinogen response to this injection was slight in Case 22, showing a maximum increase of 19 per cent in 36 hours; in Case 23 there was no increase of the plasma fibrinogen above the average control level in spite of a temperature elevation to 105°F. for 7½ hours. The leukocyte responses were similar to those described above.



FIG. 5. Plasma fibrinogen, leukocyte and temperature responses in Case 18, subjected to fever as induced, first, by high environmental temperature and, second, by the intravenous injection of typhoid vaccine.

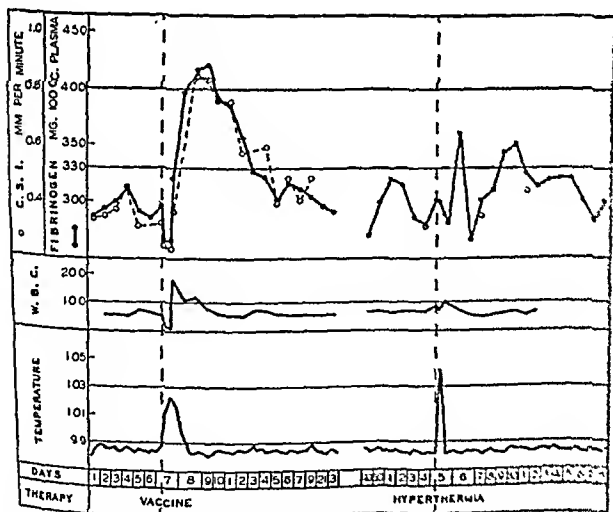


FIG. 6. Plasma fibrinogen, corrected sedimentation index, leukocyte and temperature variations in Case 9, subjected to fever as induced, first, by the intravenous injection of typhoid vaccine and, second, by high environmental temperature.

B. *High Environmental Temperature.* Four subjects, Cases 17, 18, 9 and 22, who received the intravenous injection of typhoid vaccine described above also were subjected to high environmental temperature. There was either no response of plasma fibrinogen or an irregular and moderate variation in fibrinogen concentration, as shown in Figures 4, 5, 6 and 7. Maximum increases above the average control level of 23 per cent occurred in 2 instances, Cases 18 and 9; 1

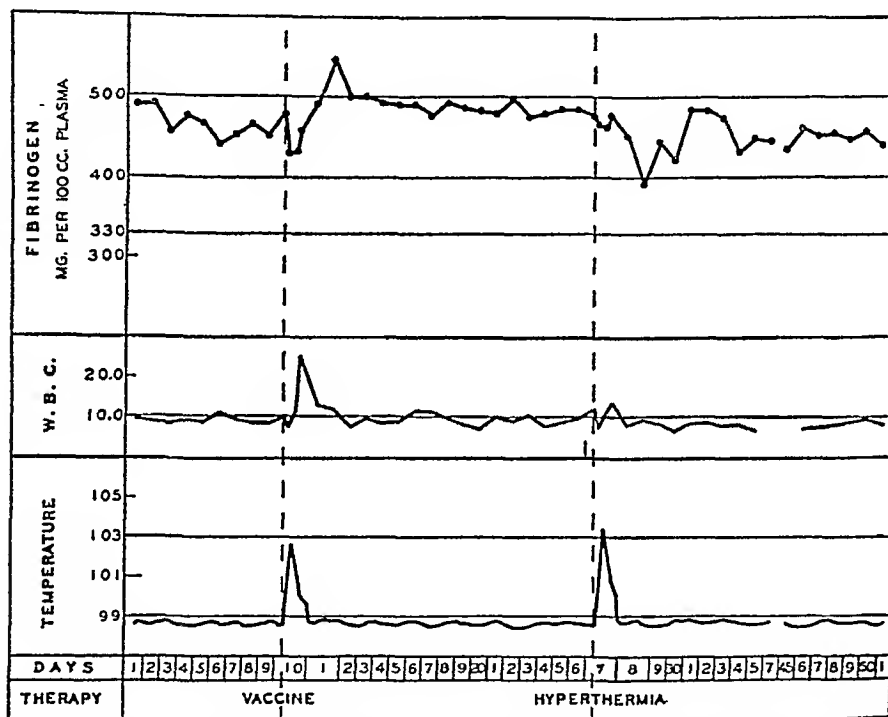


FIG. 7. Plasma fibrinogen, leukocyte and temperature responses in Case 22, subjected to fever as induced, first, by the intravenous injection of typhoid vaccine and, second, by high environmental temperature. The fibrinogen level was abnormally elevated during the control period associated with chronic gonococcal arthritis.

case showed a slight decrease, Case 22; and 1, Case 17, showed no change. Case 17 was subjected to a body temperature of from 105° to 106°F. for 5 hours; Case 18 to a temperature of from 105° to 105.4°F. for 3 hours and to above 102°F. for a total of 5 hours; Case 9 to a temperature of from 103° to 105.4°F. for 6½ hours; Case 22 to a temperature reproducing the fever induced by typhoid vaccine. The leukocyte response was of less magnitude than that observed following typhoid vaccine and no leukopenia was noted. The maximum leuko-

cytosis occurred in from 5 to 12 hours, with an average maximum value for the 4 subjects of 15,700 white cells per cu.mm.

There was no consistent or significant alteration of the serum albumin or globulins which was comparable to the changes in plasma fibrinogen observed following either the injection of typhoid vaccine or exposure to high environmental temperature.

C. Summary. Elevation of the body temperature by high environmental temperature in 4 patients produced either no fibrinogen response, a decrease in concentration or a maximum increase of only 23 per cent above the average control level. These responses are slight compared to the abnormal levels of fibrinogen usually observed in infectious diseases with similar temperature elevations. However, the injection of a single large dose of typhoid vaccine in 6 subjects with normal fibrinogen levels produced significant and prolonged elevations of fibrinogen which were not necessarily proportional to the maximum temperature rise or to the duration of fever. In 2 subjects with elevated fibrinogen levels the injection of typhoid vaccine produced either no fibrinogen increase or only a slight increase in concentration. From this direct experimental evidence and from the clinical evidence that fibrinogen may be extremely elevated in afebrile diseases it is apparent that fever per se may not cause the elevated plasma fibrinogen observed clinically. Both increased fibrinogen and fever are probably caused by factors common to the disease process.

VI. FIBRINOGEN RESPONSE TO INFECTION

The characteristics of the plasma fibrinogen response and the responses of body temperature and leukocytes were observed in certain infectious conditions and compared to the responses described above following induced fever.

The fibrinogen response to an acute respiratory infection of moderate severity without fever is illustrated by Case 24, shown in Figure 8. Normal fibrinogen values had been observed over a period of 41 weeks during health and just prior to the abrupt onset of this infection, which was evidenced by coryza, pharyngitis and moderate cough. A beta hemolytic streptococcus was cultured from the throat on two occasions during the first week. The subject did not feel ill, however,

and continued to work. This afebrile infection, with a maximum leukocytosis of only 11,400, was characterized by a progressive elevation of fibrinogen to an abnormal concentration of 400 mg. on the eighth day after the acute onset and a slow return to the previous level over a period of 2 weeks.

In a patient with fatal lobar pneumonia, Case 25, the fibrinogen response did not reach a maximum until the fifth day of the disease, after daily increases of approximately 100 mg. associated with a transient leukopenia, as shown in Figure 9.

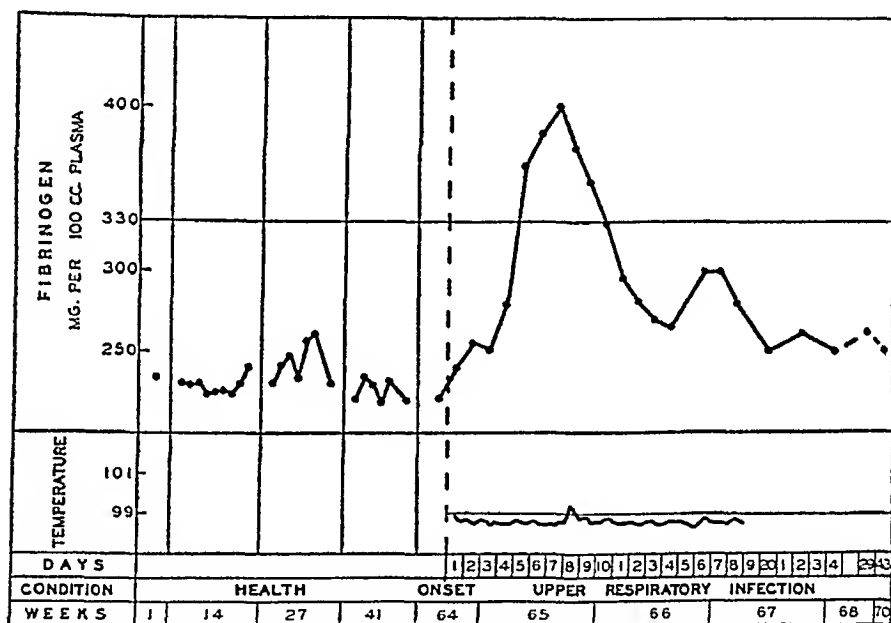


FIG. 8. Case 24. Plasma fibrinogen response following an acute upper respiratory infection of moderate severity.

The influence on the fibrinogen level of two separate infectious episodes was evident from observation of Case 26, shown in Figure 10. During the first episode the maximum temperature was 105.4°F. and the patient showed signs of an acute upper respiratory infection. For the next week the temperature remained normal and the patient was convalescent. This was followed by a second episode of pharyngitis, with 4 days of fever and subsequent recovery. Following each episode the fibrinogen response was similar, with a maximum concentration occurring 3 days after the onset of fever at a time when

the temperature already had returned to normal. A prolonged period of several weeks was required for the fibrinogen level to return to the upper limits of normal. There was no significant leukocytosis with either episode.

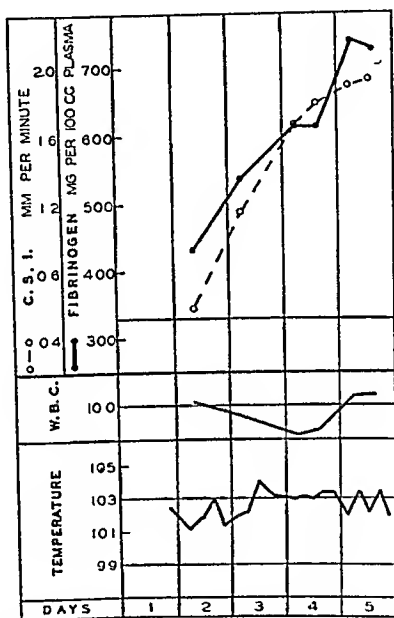


FIG. 9. Case 25. Plasma fibrinogen response and corrected sedimentation index in a fatal instance of lobar pneumonia associated with transient leukopenia.

Significant variation of the fibrinogen concentration within the range of normal values was observed on two separate occasions in Case 27, associated in both instances with mild attacks of pleuritis but not accompanied by fever or leukocytosis. The fibrinogen level observed for 12 months varied from 220 mg. to 260 mg. during periods of health, but in the week following the first attack of pleuritis the fibrinogen concentration increased to 320 mg. Six months later, in

the week following the second attack, the fibrinogen increased to 340 mg. In each instance the value slowly returned to the previous level during several weeks.

That the fibrinogen response may be maximum despite failure of the leukocytic response was observed in a fatal instance of acute agranulocytosis, Case 28. In this patient, observed for 3 days before death, the leukocytes varied from 275 to 934 per cu.mm. with complete

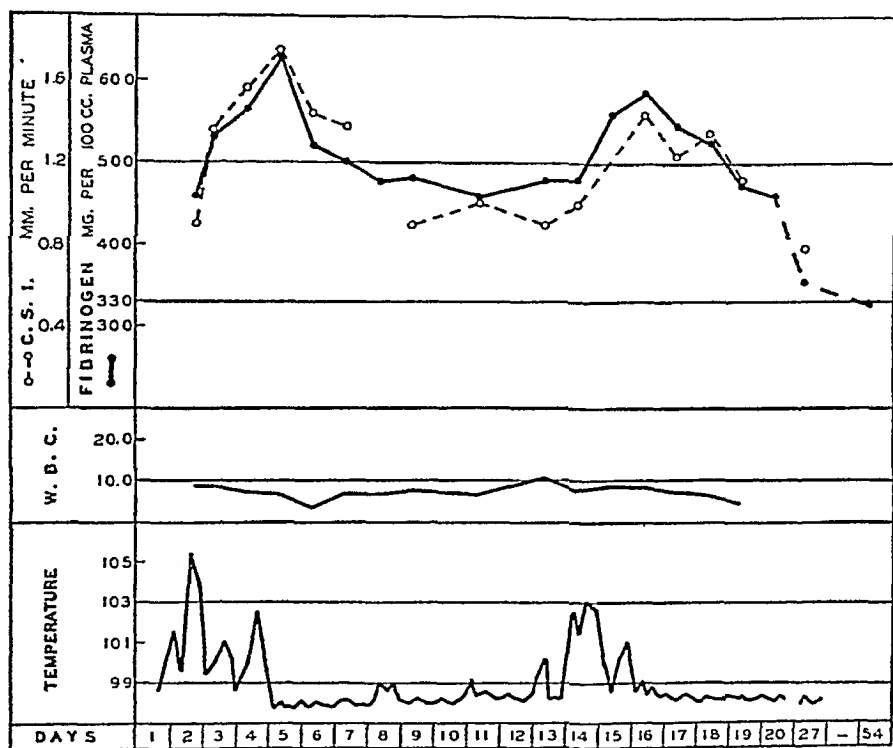


FIG. 10. Case 26. Plasma fibrinogen response and corrected sedimentation index following two separate infectious episodes.

absence of granulocytes. The temperature was elevated to between 103° and 105°F. The pharynx showed gangrenous necrosis. Four fibrinogen values varied from 830 mg. to 1,130 mg. per 100 cc. of plasma, with an average concentration of 1,020 mg.

In *chronic infections*, as evident in Cases 22 and 23 with gonococcal arthritis, the fibrinogen response is one of prolonged abnormal elevation frequently associated with normal body temperature and leukocytes (11, 28).

In *summary*, it is evident that the plasma fibrinogen response to acute infection may show no increase on the first day of the disease and may require several days or longer to obtain a maximum concentration. This point of highest concentration on the curve may occur after the decrease of fever and the diminution of clinical signs of infection. The return to a normal fibrinogen level may require a period of several weeks after the symptoms and signs of infection have terminated. The fibrinogen response in infectious disease may be present without fever or leukocytosis and despite a failure of the leukocyte response.

VII. FAILURE OF THE FIBRINOGEN RESPONSE

In most infectious diseases and in most conditions associated with tissue destruction or inflammation the plasma fibrinogen is significantly elevated during the course of the disease (12, 19, 28, 66, 74). Although leukocytosis and fever may be slight or entirely absent, the effectiveness of the stimulus to fibrinogen production afforded by slight acute infection or by chronic low grade infection of moderate degree is usually indicated by the resultant material increase in fibrinogen concentration. It is possible that the lack of fibrinogen response occasionally observed (28, 74) in certain infectious diseases, such as mumps, measles and sometimes in influenza, may be due to inadequate stimulus for fibrinogen production.

Experimentally, the production of fibrinogen has been delayed or even completely inhibited by procedures or agents causing severe liver destruction or impairment of function (9, 19, 24, 41, 49, 53, 67, 70, 80, 81). The observations of Foster and Whipple (19) and of others (55, 70, 72) on laboratory animals clearly demonstrate that moderate liver damage itself is a stimulus to fibrinogen production. With more extensive destruction of the liver, however, there occurs a failure of the fibrinogen regeneration. Under these circumstances, moreover, the addition of a further known stimulus to fibrinogen production, such as a turpentine abscess, is ineffective (19).

Similar phenomena also are observed clinically in human subjects. Thus a material elevation in fibrinogen concentration is found in instances of moderate liver damage such as occur in catarrhal jaundice, obstructive jaundice and hepatitis from infection, drugs, neoplasms

and passive congestion (5, 10, 15, 21, 28, 46, 74). In instances of overwhelming or extensive liver destruction, however, such as occur in acute yellow atrophy, cirrhosis, amyloidosis, fatty degeneration and hepatitis from various causes, the usual fibrinogen responses may be lacking even in the presence of infection (10, 15, 21, 28, 42, 51, 52, 58, 74, 83). Fibrinopenia, a concentration below 100 mg. per 100 cc. or the complete absence of fibrinogen from the plasma with hemorrhagic diathesis has been correlated with extreme liver injury (58); in other instances the liver damage has been less severe (42) or has not been studied at postmortem (62). Rarely, complete absence of fibrinogen with bleeding has been observed as a congenital defect with no evident liver disease (47, 57, 63, 66).

The influence of the degree of liver damage in determining the degree of fibrinogen response is suggested by the clinical observation that in certain instances of tuberculosis, a condition generally associated with abnormally elevated fibrinogen values, normal or low fibrinogen concentrations have been observed in terminal cases which on postmortem examination show extensive liver damage (52, 58).

For these reasons the lack of a fibrinogen response in the presence of a *severe* infection may suggest liver disease. The obvious clinical manifestations of liver injury may be lacking during life, however, as illustrated by certain of the following cases, showing a lack of fibrinogen response and, at autopsy, liver damage of variable degree. In the first 3 patients, Cases 29, 30 and 31, severe infection should have provided an adequate stimulus to fibrinogen production, as should the hepatitis of the fourth patient, Case 32.

Case 29. A male of 18 was observed for 6 weeks before his death from subacute bacterial endocarditis due to the streptococcus viridans. During life the patient showed a normal galactose tolerance test, normal urobilinogen concentrations in the urine on two occasions, an icteric index between 10 and 22 and a positive Takata-Ara reaction. There was no terminal drop in blood sugar or blood urea nitrogen, which 5 days before death were observed to be 120 mg. and 37 mg., respectively. Blood cultures were positive for the streptococcus viridans on four occasions. Throughout the period of observation the patient's temperature was septic in type; the maximum leukocytosis was 13,800 per cu. mm. with a terminal leukopenia. The fibrinogen concentration, however, was within the normal range on

6 occasions, below normal on 5 occasions, and varied from 140 mg. to 300 mg. per 100 cc. of plasma with an average concentration of 200 mg. Autopsy revealed congenital and rheumatic valvular disease of the heart, cardiac enlargement, terminal lobar pneumonia, multiple pulmonary infarctions and acute endocarditis. Culture of the heart valves showed streptococcus viridans; cultures of the heart's blood, lungs and pleura showed pneumococcus Type XIX. Histologically the liver presented an increase in fibrous tissue, nodular regeneration, a proliferation of fibrous tissue and bile ducts in the portal areas, with monocytic infiltration. The liver cells, however, appeared normal, there was no extreme passive congestion and a pathological diagnosis was made of healed cholangitis without acute liver necrosis.

Case 30. A male of 44 with a history of acute and chronic alcoholism was observed for 3 days before his death from Friedlander's bacillus pneumonia. There was no clinical evidence of liver disease during life. The corrected sedimentation index of Rourke and Ernstene decreased from a slightly elevated level of 0.6 mm. per minute on the first day to 0.1 and 0.4 mm. per minute on the second and last days of life. Plasma fibrinogen observations were not made. These data on the sedimentation rate, however, suggest that the plasma fibrinogen was not elevated above normal concentrations. At autopsy *no fibrin* was recognized in sections of the consolidated lung. The liver was light yellow in color, weighed 3,150 grams and microscopically showed advanced fatty (alcoholic) and granular degeneration of the parenchyma.

Case 31. A male of 51 was observed for 4 weeks before his death from pernicious anemia, which was complicated by advanced subacute combined degeneration of the spinal cord, "paralytic bladder" with cystitis and gangrenous decubitus of the sacrum. During the last 3 weeks of life the temperature was septic in type. Blood cultures showed no growth on two occasions. There was no elevation of the icteric index or other sign of liver disease and no terminal decrease in the blood non-protein nitrogen. The plasma fibrinogen was normal, varying from 200 mg. to 260 mg. on three occasions during the last 2 days of life. At autopsy the liver showed a moderate degree of passive congestion with occasional small areas of acute focal necrosis. In addition to cystitis and gangrenous decubitus there was infarction of the right lower lobe of the lung.

Case 32. A male of 37 received a course of 14 injections of neoarsphenamine during a period of 4 months preceding his death, which occurred 7

weeks after the last injection and 3 weeks after the first development of jaundice. Clinically the patient presented the signs of tabes dorsalis, optic atrophy, rapidly progressive jaundice, decreasing liver size and coma. The urine contained large amounts of bile and leucine and tyrosine crystals. Shortly before death the blood urea nitrogen was 45 mg., with a total non-protein nitrogen of 60 mg. The plasma fibrinogen remained at normal or subnormal levels during the last 4 days of life, varying from 180 mg. to 240 mg. per 100 cc. of plasma. At autopsy the liver weighed 1,720 grams and histologically showed diffuse acute toxic damage to liver cells, as evidenced by fatty degeneration. This acute change was apparently superimposed on acute cholangitis and biliary infectious cirrhosis.

In *summary*, the plasma fibrinogen concentration apparently failed to increase above normal levels in 3 patients with severe infection and 1 patient with hepatitis. Clinically, there was no evidence for liver disease in 2 of the 4 patients, but at postmortem examination in each instance there was a significant liver damage, which varied in severity and in the degree of anatomical involvement. In no instance, however, was the liver disease of the extreme severity usually associated with failure of fibrinogen regeneration. It is impossible to establish a causal relationship between the fibrinogen level and the liver disease in these patients. These cases suggest, however, that liver injury of variable degree, which may not be demonstrable clinically, may be associated with a failure of plasma fibrinogen to respond.

VIII. DISCUSSION

For any one individual the fibrinogen level during health is relatively constant and is not altered by short periods of fasting, by food ingestion, by rest or brief violent exercise. For a series of normal subjects, however, the range of fibrinogen concentrations from 190 mg. to 330 mg. is materially greater than the variations in level occurring in any one subject during health. A diet deficient in protein will eventually produce subnormal fibrinogen concentrations, but such a diet, and nutritional deficiency in general, do not prevent the response of fibrinogen to infection. The low fibrinogen level observed in pernicious anemia is apparently the result of nutritional deficiency and is not increased by remission of the anemia induced by liver extract unless animal protein is added to the diet. The fibrinogen con-

centrations in normal subjects and in patients with nutritional deficiency are increased moderately, but usually within normal limits, by diets rich in animal protein derived from any one of several sources. The influence of diet on the fibrinogen level is only moderate in degree and has no apparent clinical significance.

Considered broadly, since an elevation of fibrinogen concentration is so common a reaction to such a variety of disease conditions, it is classified as a nonspecific response of the organism and is comparable, therefore, to other nonspecific reactions, such as hyperpyrexia, leukocytosis and tachycardia (12, 13). The variations in fibrinogen level may be entirely independent of variations in body temperature, leukocytes and the other plasma proteins. The fibrinogen response may be observed more frequently than either fever or leukocytosis in many disease processes, such as acute and chronic infections and malignant neoplasms (28, 52). The fibrinogen level is often the last to return to normal (11, 54). Therefore, increase in fibrinogen concentration is usually a more sensitive indicator than other responses of the presence of tissue injury, especially that produced by low grade chronic infection.

As an index of the time relationship of the fibrinogen response to the stimulus produced by infection, the intravenous injection of typhoid vaccine is probably a more readily defined stimulus than occurs in clinical instances of infection. In general, the response to the injection of typhoid vaccine was characterized by three phases: an initial drop or no change in concentration within 12 hours after injection, when leukocyte and temperature responses were at a maximum; a secondary rise to a maximum value within 36 to 72 hours; and a gradual return to the original level over a period of 4 to 10 days, depending on the dose of vaccine employed.

In clinical instances of infection, marked by a sharply defined onset, the fibrinogen response is apparently similar to that following the intravenous injection of typhoid vaccine. On the first day of an *acute infection*, even in the presence of fever and leukocytosis, the fibrinogen level or sedimentation rate frequently is normal (36, 54, 56). The maximum fibrinogen elevation, to levels of from 400 mg. to 800 mg. or occasionally to higher concentrations, may not occur for several days when the leukocytes and the temperature already

may have returned to normal. During convalescence the fibrinogen concentration usually decreases slowly and progressively to normal over a period of from 2 to 8 weeks (54) after the leukocytes and temperature have returned to normal and after the disappearance of all symptoms and signs of the disease process. Exacerbations of the infection or other infectious complications produce further increases in fibrinogen after a variable lag period. Usually the fibrinogen concentration is elevated significantly above normal levels. In certain mild infections, however, an increase of 100 mg. in concentration may not result in a fibrinogen value beyond normal limits, but nevertheless may represent a fibrinogen response of pathological significance for the individual. Such a moderate response would not be detected without a previous knowledge of the fibrinogen level during health. From a practical standpoint the fibrinogen concentration is not artificially altered by the usual variations of a subject's diet or muscular activity. It is not altered by the variety of functional disorders. An increase in concentration of 100 mg. or more usually constitutes a fibrinogen response, therefore, frequently signifying the presence of some organic disease process.

In contrast to acute infection, *chronic infection* and chronic tissue destruction in general produce prolonged and significant elevation of fibrinogen, usually to levels of from 400 mg. to 800 mg. and occasionally to higher levels. This response may or may not be associated with increases in leukocytes or body temperature. Normal pregnancy is the only "physiological" condition associated with increased plasma fibrinogen concentrations to values of from 350 mg. to 450 mg. (62).

Diagnostically, therefore, an abnormally elevated plasma fibrinogen concentration in the absence of pregnancy is laboratory evidence for an organic disease process which may be present at the time of observation or which may have terminated several days or weeks before. Because of the lag in the fibrinogen response to acute infection and because of protracted elevation in chronic infection the fibrinogen level, as indicated by the sedimentation rate, has served as an aid in distinguishing between acute and chronic infectious processes, such as acute appendicitis and chronic pelvic inflammation (22, 36, 77, 82). Since the fibrinogen response is delicate and nonspecific it

may not be a reliable guide, however, either to the kind or to the extent of tissue damage. A normal fibrinogen concentration is evidence usually against the diagnosis of certain chronic infections, malignant disease and a variety of organic conditions which produce tissue destruction and inflammation. A normal fibrinogen concentration, however, is compatible with a diagnosis of many functional allergic, metabolic and degenerative diseases.

The fibrinogen concentration, as indicated by the sedimentation rate, has served largely as a laboratory aid in determining the *progress* and *therapeutic results* of certain potentially serious chronic infections, especially tuberculosis, rheumatic fever, arthritis and gonococcal infections (2, 11, 20, 22, 27, 50, 59, 64, 71, 78, 82). In these and similar conditions the most delicate index of disease activity is essential. In general, the fibrinogen concentration remains significantly elevated as long as disease is present and "active"; the concentration increases with exacerbations or complications and decreases with remissions and cure. In many other conditions which are not potentially dangerous it is unnecessary to confine a patient to bed because of an abnormally elevated plasma fibrinogen level.

The occasional failure of fibrinogen to respond in severe infection may be a poor prognostic sign and may suggest inadequate liver function. Under these conditions liver disease may not be demonstrable clinically, but at postmortem examination the liver may show damage of variable extent and degree. The lack of fibrinogen response in certain infections of mild or moderate degree, such as the virus diseases, may be due to inadequate stimulus to fibrinogen production, since these processes generally are nonpyogenic. The corrected sedimentation index of Rourke and Ernstone correlated roughly with fibrinogen concentration in the instances where it was employed. A detailed discussion of the sedimentation rate is presented in the next paper in this Journal.

IX. CONCLUSIONS

1. The plasma fibrinogen concentration in normal human subjects is relatively constant for any one individual and is not significantly influenced by the conditions of fasting, food ingestion, rest and short violent exercise. The limits of concentration observed for normal

adult males and females are from 190 mg. to 330 mg. per 100 cc. of plasma, with an average level of approximately 250 mg.

2. In uncomplicated and untreated cases of pernicious anemia that have received inadequate dietary protein, the fibrinogen concentrations vary from below normal to normal levels with a lower average concentration than that observed in healthy subjects.

3. Nutritional deficiency in pernicious anemia, in scurvy and in pellagra does not prevent an increase of fibrinogen above normal in the presence of infection.

4. In normal subjects the daily ingestion of animal protein in moderate or large amounts produces a moderate fibrinogen response, usually within normal limits.

5. Remission from pernicious anemia does not result in an increase of plasma fibrinogen when the diet is deficient in protein, but is accompanied by an increase of fibrinogen from subnormal levels to normal when the diet contains 50 grams daily of protein derived from any one of several animal sources.

6. Fever induced by high environmental temperature causes either no elevation or only slight and irregular elevation of plasma fibrinogen, whereas the intravenous injection of typhoid vaccine causes both fever and a significant and prolonged elevation of the fibrinogen level. Fever per se probably is not the cause of the abnormally increased fibrinogen concentrations observed in many febrile diseases.

7. In infectious disease the fibrinogen response may be independent of the temperature and the leukocyte changes, and may occur despite a failure of the leukocyte response.

8. A failure of fibrinogen to increase above normal in instances of severe infection may be a poor prognostic sign and may suggest the presence of significant liver damage even though clinical signs of liver disease are lacking.

9. Elevation of plasma fibrinogen is one of the most common non-specific responses of the body to a variety of disease conditions and is comparable to other nonspecific reactions of leukocytosis and hyperpyrexia. The fibrinogen response may vary independently of variations of other plasma proteins.

10. Knowledge of the fibrinogen concentration may serve as a clinical aid in the diagnosis and in the management of disease conditions.

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SEDIMENTATION RATE OF ERYTHROCYTES

INFLUENCE OF TECHNICAL, ERYTHROCYTE AND PLASMA FACTORS AND QUANTITATIVE COMPARISON OF FIVE COMMONLY USED SEDIMENTATION METHODS

THOMAS HALE HAM, M.D., AND FANNY C. CURTIS, A.B.

*From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard),
Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston*

I. Introduction.....	448
II. Historical.....	449
III. Physicochemical Forces Affecting the Sedimentation Rate.....	450
IV. Factors Affecting the Sedimentation Rate as Employed Clinically.....	452
A. Technical Factors.....	452
1. Methods of Timing.....	455
2. Duplication Accuracy.....	456
3. Height of Blood Column.....	458
4. Internal Diameter of Sedimentation Tube.....	458
5. Anticoagulants.....	459
6. Effect of Standing.....	462
7. Temperature.....	463
8. Inclination of Tube.....	464
9. Discussion and Summary.....	464
B. Erythrocyte Factors.....	465
1. Erythrocyte Concentration.....	465
2. Erythrocyte Size.....	472
3. Discussion and Summary.....	475
C. Sedimentation Rate as a Measure of Fibrinogen, Serum Globulins and Lipoids.....	476
1. Whole Blood Sedimentation Rate as a Measure of Fibrinogen.....	477
2. Defibrinated Blood Sedimentation Rate as a Measure of Serum Globu- lins and Lipoids.....	492
3. Discussion and Summary.....	492
V. Quantitative Comparison of Sedimentation Methods.....	495
A. Wintrobe-Landsberg Method.....	495
B. Westergren Method.....	497
C. Linzenmeier and Cutler Methods.....	500
D. Discussion and Summary.....	501
VI. Clinical Interpretation of Erythrocyte Sedimentation Rate.....	502

VII. Conclusions.....	505
VIII. Appendix—Methods.....	507
A. Plasma Proteins and Cholesterol.....	507
B. Hematology.....	507
C. Whole Blood Sedimentation.....	508
D. Defibrinated Blood Sedimentation.....	510
Bibliography.....	511

I. INTRODUCTION

Following the investigations of Fahraeus in 1918 and 1921 (22, 23, 24) on the sedimentation rate of erythrocytes suspended in plasma, the sedimentation "test" has been employed as a laboratory aid in all branches of medicine (1, 30, 36, 60, 67, 91, 114, 120). It has served as a diagnostic test for the presence of inflammation or in distinguishing between acute and chronic infectious processes (51, 112, 120). It has been utilized as a guide to the progress and treatment of chronic infections, especially tuberculosis, rheumatic fever and arthritis (2, 16, 21, 29, 30, 60, 73, 101, 114, 120).

Although much is known about the factors influencing the sedimentation rate, the clinical significance of sedimentation data frequently is uncertain. A multiplicity of methods has caused confusion by the lack of standardization of technique and by the difficulty of converting the units of one method into those of another. In general there has been a failure to recognize that the sedimentation rate may correlate in a roughly quantitative manner with the concentration of certain constituents of blood plasma, especially plasma fibrinogen (4, 21, 31, 116).

The object of this communication is to discuss the meaning of the sedimentation test and to evaluate its place in clinical medicine as a laboratory aid. For this purpose the influence of certain technical and erythrocyte factors was reinvestigated. The sedimentation rate of *whole blood* and the sedimentation rate of *defibrinated blood* were studied primarily to determine their value and limitations as indirect methods for estimating the concentration of certain constituents of plasma or serum. Accordingly, the correlations between these sedimentation rates and the concentrations of plasma fibrinogen, serum globulins and lipoids were studied quantitatively. The

degree of correlation between the sedimentation rate and plasma fibrinogen was investigated for five commonly employed sedimentation methods for whole blood, namely, those of Linzenmeier (69), Westergren (113), Cutler (15), Rourke-Ernstene (94) and Wintrobe-Landsberg (121). The mechanism responsible for the variations in the stability of erythrocyte suspensions was not studied.

II. HISTORICAL

The settling velocity of erythrocytes was first applied as a clinical test by Fahraeus in 1918 (23) in the study of pregnancy. In the same year he published preliminary observations of the factors influencing the stability of erythrocyte suspensions (22). In two subsequent monographs (24, 25), published in 1921 and 1929, the same author made a classical review of the ancient and modern literature and demonstrated that acceleration of the sedimentation rate was correlated with changes in the concentration of certain plasma constituents, especially fibrinogen or serum globulins, associated with variable degrees of erythrocyte aggregation. Gram in 1921 (34) came to similar conclusions. Linzenmeier in 1920 (69) introduced his sedimentation technique and in the same year Westergren, an associate of Fahraeus, described the method which has become the standard for Europe (113, 114, 115). Since 1921 several thousand publications have appeared, dealing primarily with the clinical application of the sedimentation rate. These have been reviewed recently by Reichel (91), especially with reference to the Westergren technique, and by others (30, 60, 67, 114, 120). Lundgren in 1927 (71) made studies of the physical nature of sedimentation. Westergren (114), Gram (35), Rourke and Ernstene (94) and others (10, 121) have investigated the influence of erythrocyte concentration and several authors have introduced methods in which correction for the erythrocyte concentration is an integral part of the technique. The correlation of the sedimentation rate with fibrinogen and with serum globulins has been further defined by Ernstene (21), Bendien and Snapper (4), Westergren and coworkers (116), Kylin (64) and others (31, 37, 45, 46, 82, 125). Bendien, Neuberg and Snapper (3) have demonstrated the influence of erythrocyte size on the sedimentation rate.

III. PHYSICOCHEMICAL FORCES AFFECTING THE SEDIMENTATION RATE

Erythrocytes suspended in plasma or serum settle because their density is greater than the density of their medium (3, 24, 49, 83). The settling is resisted by the viscosity of the medium. Stokes' law, which pertains to an ideal system of spherical particles suspended in a medium of infinite extension (12, 65, 88, 103), has been used to explain the variations in the settling velocity of erythrocytes (3, 24, 25, 60, 91). However, Stokes' law cannot be applied mathematically to an erythrocyte-plasma system without modification because of two outstanding differences from the ideal system. First, the extent of the medium is limited by the relatively large total volume of particles and by the confining dimensions of the tube. Second, the erythrocytes form nonspherical aggregates of inconstant size and shape.

Erythrocytes suspended in plasma or serum always form aggregates or rouleaux varying in size from a few cells per aggregate to particles which are visible macroscopically (24, 92, 97, 100). In general the sedimentation rate increases in proportion to the size of the erythrocyte aggregates (24, 25). This is explained by the geometric law that the larger the volume of a particle the smaller is the relative surface. Accordingly, the downward force, which is governed by the total mass of the particle, increases more rapidly than the retarding force, which is a function of the surface area exposed to the medium. The degree of erythrocyte aggregation and the rate of sedimentation have been demonstrated to correlate roughly with the concentration of certain plasma constituents, especially plasma fibrinogen and serum globulins (4, 24). The stability of erythrocyte suspensions probably is related to the colloidal state and to the stability of plasma or serum itself (3, 22, 24, 25, 52, 68, 104). However, the exact physicochemical mechanism causing erythrocyte aggregation is not established, nor is it established that the various globulins of the blood are directly or causally related to rouleaux formation (19). This subject has been studied by many investigators but is beyond the scope of this communication (3, 20, 22, 24, 52, 68, 70, 79, 80, 81, 89, 90, 99, 104, 106, 108, 118). It is apparent, however, that any substance of the plasma or the serum

which directly or indirectly affects the degree of erythrocyte aggregation will alter the stability of the suspension and, therefore, the sedimentation rate.

When an erythrocyte suspension is placed in a vertical sedimentation tube, progressive increase in the size of the erythrocyte aggregates takes place for a variable period of time (24, 71). This results in an initial period of progressive acceleration of the sedimentation rate, lasting until a period of constant fall is attained coincident with the development of the maximum size of the aggregates.

The settling of erythrocyte aggregates in the confining tube results in upward displacement of the medium (24, 71, 61). Accordingly, the particles do not fall through a stationary fluid and the upward current causes the *apparent* settling velocity to be less than the actual velocity of fall through the medium. The greater the concentration of erythrocytes, the greater will be the volume of plasma displaced and consequently the greater the retardation of the apparent settling velocity. This is probably the major mechanism underlying the variations in the sedimentation rate produced by alteration of the concentration of erythrocytes in suspension. In tubes of internal diameter of 2.0 mm. or less, retardation or irregularity of the settling velocity is observed (91, 111). Probably this is due to the fact that the internal circumference of a small tube is disproportionately large in relation to its area of cross section and, therefore, produces a relatively great frictional surface at the wall of the tube with consequent retardation of the sedimentation. Increase in the height of the original column of blood produces acceleration of the erythrocyte settling velocity (26, 121). It is probable that the higher the column of blood the less is the retarding effect of displacement currents on the upper portion of the erythrocyte column. Deviation of the sedimentation tube from the vertical by only a few degrees causes a striking acceleration in the sedimentation rate (71, 88, 121). According to the observations of Ponder (88) and of Lundgren (71), under these circumstances the plasma streams rapidly along the upper side of the tube and the erythrocytes are thus subjected to less hindrance from the displacement currents, as in the familiar example of the angle centrifuge.

A characteristic of all sedimentation rates is a final progressive

retardation or "packing" following the period of constant settling velocity (1, 13, 24, 71, 94). Due to the accumulation of erythrocytes at the bottom of the tube the effective "bottom" of the column of erythrocytes is raised above the bottom of the glass tube and, as with a short original column of blood, displacement currents have a greater slowing effect.

In summary, erythrocytes suspended in plasma or serum settle by virtue of their greater density. The wide range of sedimentation rates observed clinically, however, results primarily from the direct or indirect influence of constituents of plasma or serum by producing great variation in the degree of erythrocyte aggregation. Although the major factor affecting the settling velocity is the rouleaux formation, the sedimentation rate is modified by other factors, such as the viscosity of the medium and its limited extent.

IV. FACTORS AFFECTING THE SEDIMENTATION RATE AS EMPLOYED CLINICALLY

From the previous discussion it is apparent that Stokes' law cannot be applied mathematically to the sedimentation rate of blood samples. It is necessary, therefore, to consider arbitrarily the various factors affecting the sedimentation rate. The influence of variations in technique and in erythrocytes was evaluated before investigating the correlation between the sedimentation rate and the constituents of plasma or serum. All *methods* employed in these experiments are described in the *appendix*. The sedimentation technique of Rourke and Ernstene (94) was selected as the standard procedure.

A. Technical Factors

The practical importance of recognizing the influence of technical variations is at once apparent when a single blood sample is examined by several commonly employed sedimentation methods, as in the following experiment and as shown by others (1, 39, 46).

A 15-cc. sample of venous blood was withdrawn from a patient with rheumatic fever and appropriate amounts for the determination of the sedimentation rate were treated as directed by the following authors: Linzenmeier, Westergren, Cutler, Rourke-Ernstene and Wintrobe-Landsberg. The sedimentation rates as recorded by each method were as follows: Linzenmeier, 29 minutes to reach the 18

mm. mark; Westergren, 50 mm. in 1 hour; Cutler, "vertical curve" or 23 mm. in 1 hour; Rourke-Ernstene, 1.75 mm. per minute (corrected); Wintrobe-Landsberg, 45 mm. in 1 hour (corrected). Even with the application to each sedimentation curve in Figure 1 of the

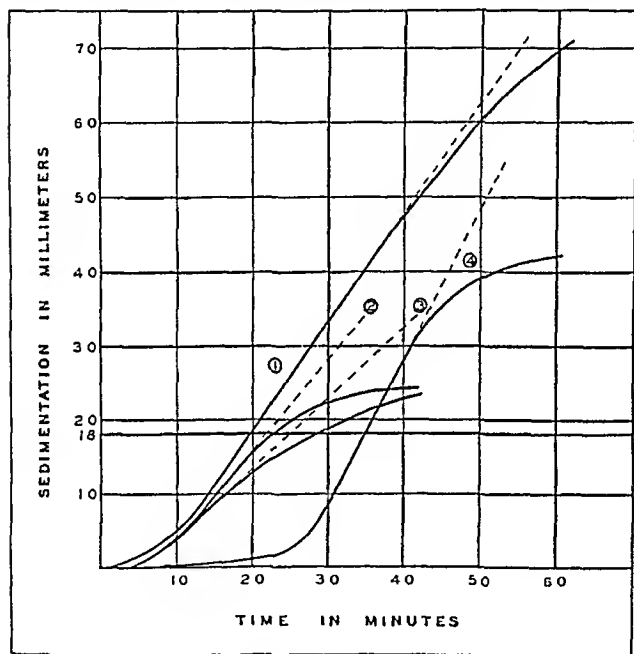


FIG. 1. SEDIMENTATION CURVES OF
SEDIMENTATION METHODS OF: (1) SPECIMEN BY THE
(2) ROURKE-ERNS. 3) LINZENMEIER;
Erythrocyte cell volume 42.8 per cent

slope method of timing described below, the results expressed in millimeters per minute differed materially and were as follows: Linzenmeier, 0.95; Westergren, 1.47; Cutler, 0.95; Rourke-Ernstene or Wintrobe-Landsberg, 2.02 (uncorrected).

Comparison of the above five sedimentation methods as sum-

TABLE 1
Comparison of the Technique Employed in Fine Sedimentation Methods

	AUTHOR AND YEAR				
	Rourke-Ernstene, 1930 (94)	Wintrobe-Landsberg, 1935 (121)	Westergren, 1920 (113)	Linzenmeier, 1920 (69)	Cutler, 1926 (13)
<i>Sedimentation</i>					
Tube Length	120 mm.	120 mm.	300 mm.	65 mm.	70 mm.
Internal Diameter	4.0 mm.	2.5 mm.	2.5 mm.	5.0 mm.	5.0 mm.
Graduations	0-100 mm.	0-100 mm.	0-200 mm.	0.18 mm.	0-50 mm.
Height of Blood Column	100 mm.	100 mm.	200 mm.	50 mm.	50 mm.
<i>Anticoagulant</i>					
Amount	Heparin, 15 per cent	Dry Oxalate Mixture*	Sodium Citrate, 3.8 per cent	Sodium Citrate, 5 per cent	Sodium Citrate, 3 per cent
	0.013 cc. in 3 cc. blood	For 5 cc. blood: solid potassium oxalate 4 mg., and solid ammonium oxalate 6 mg.	0.2 cc. in 1.0 cc. mixture	0.2 cc. in 1.0 cc. mixture	0.1 cc. in 1.0 cc. mixture
Concentration (mg. per 100 cc. mixture)	65	200	760	1000	300
Dilution of Blood (per cent)	0.4	0	20	20	10
Method of Timing Sedimentation Rate Units	Slope of period of constant fall*	Distance settled in 1 hour	Distance settled in 1 hour	Time to settle to 18 mm.	Graph of curve and Distance settled in 1 hour
	Millimeters per minute	Millimeters	Millimeters	Minutes	Slope of curve; and millimeters
Correction for Erythrocyte Concentration	Required	Required	Optional (35) (114) Seldom used	None	None†
Normal Range	0.05-0.40 mm. per min.	0-9 mm.—Men 0-20 mm.—Women	1-3 mm.—Men 4-7 mm.—Women	200-600 min.	"Horizontal Line" and 2-8 mm.—Men 2-10 mm.—Women
Abnormal Range					
Slight	0.4-0.6 mm. per min.	9-15 mm.—Men 20-25 mm.—Women	8-15 mm.	100-200 min.	"Diagonal Line"
Moderate	0.6-1.0 mm. per min.	15-30 mm.	15-40 mm.	60-100 min.	"Diagonal Curve"
Extreme	2.0-2.5 mm. per min.	35-50 mm.	80-110 mm.	15-30 min.	"Vertical Curve"

* See Appendix.

marized in Table 1 shows great variation in methods of timing, height of blood column, dimensions of tubes and anticoagulants. Since the influence of some of these variables on the settling velocity is uncertain or controversial these factors were reinvestigated. In the experiments described below, employing the Rourke-Ernstene method, the effect of any one technical factor was observed with all other variables maintained constant. Blood samples were employed showing both normal and a wide range of abnormal sedimentation rates. Since the concentration of erythrocytes was not a variable in these experiments, no correction for the erythrocyte concentration was required.

*1. Methods of Timing.*¹ The sedimentation rate was determined on three samples of the same blood specimen adjusted so that the erythrocyte cell volumes were, respectively, 32.1, 46 and 52.3 per cent. Adjustment was made by the addition or removal of plasma. The data are recorded graphically in Figure 2 in the form of curves which, like *all* curves expressing the sedimentation rate of erythrocytes, show that a constant settling velocity occurs only during the second period, BC (94). In the period AB the velocity is increasing; in the period CD it is decreasing. Comparable portions of a variety of curves may be measured, therefore, by observing the sedimentation rate during the period of constant fall. This may be done by *calculating the slope* of this period from a graph of the curve, as described by Lundgren in 1927 (71) and by Rourke and Ernstene in 1930 (94) (see appendix). The sedimentation velocity is reported in millimeters per minute or per hour.

In the *graphic method* of Cutler (15) the distance of fall observed at five-minute intervals is plotted on a graph for one hour. Although Cutler usually employs a description of the graph of the sedimentation curves, quantitative measurement of the slope during the period of constant fall also is used (17). In the *distance method* of timing employed by Westergren (113), Wintrobe and Landsberg (121) and also used by Cutler (13), the sedimentation rate is determined by the distance of settling at the end of a specified time interval, e.g., millimeters in one hour. In the *time method* of Linzenmeier (69),

¹ See also Section V.

the sedimentation rate is measured by observing the time required for the column of erythrocytes to settle a specified distance, e.g., minutes for a sedimentation of 18 mm. The two latter methods always include the variable first period and indeterminate portions of one or both of the subsequent two periods. Such methods may not measure necessarily comparable portions of different sedimentation curves. For this reason, in all experiments reported below the

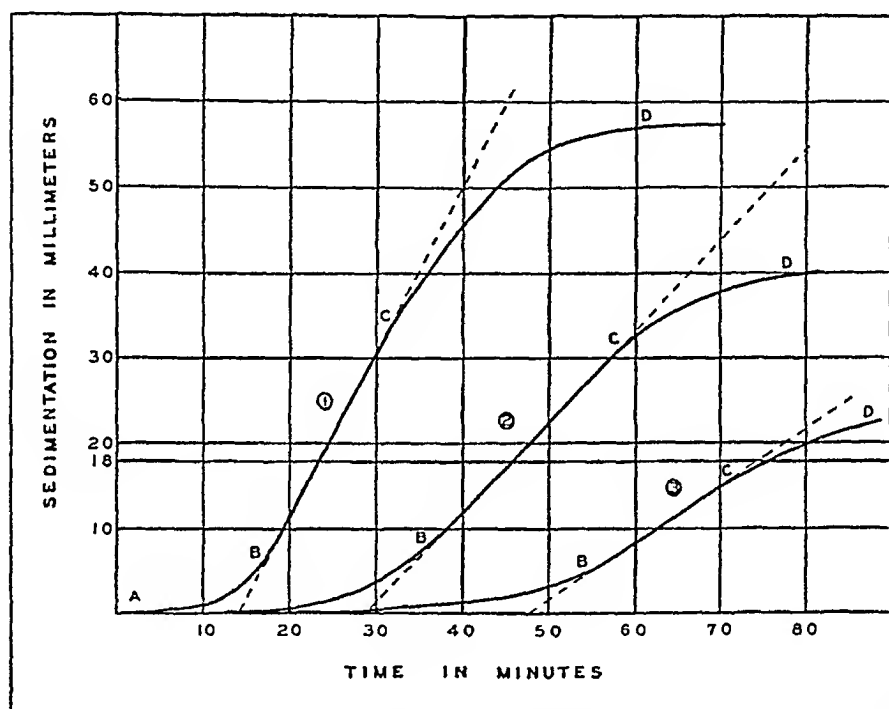


FIG. 2. SEDIMENTATION CURVES OF SAMPLES OF THE SAME BLOOD SPECIMEN IN WHICH THE ERYTHROCYTE CELL VOLUME WAS ADJUSTED TO 32.1, 46 AND 52.3 PER CENT FOR CURVES 1, 2, 3, RESPECTIVELY

The uncorrected sedimentation rates of line BC for each curve, in millimeters per minute: Curve 1—1.95; Curve 2—1.06; Curve 3—0.67. The corrected sedimentation index, in millimeters per minute: Curve 1—1.00; Curve 2—1.12; Curve 3—1.05.

slope method of timing of the Rourke-Ernstene technique was employed.

2. Duplication Accuracy. The accuracy of observation of the sedimentation rate and the erythrocyte cell volume per cent on duplicate samples of the same blood specimen was investigated in 11 experiments. The data are shown in Table 2. The average difference between duplicate determinations of the sedimentation rate was 2

TABLE 2

Accuracy of Duplicating the Uncorrected Sedimentation Rate and the Erythrocyte Cell Volume Per cent

DUPLICATE DETERMINATIONS Uncorrected Sedimentation Rate (mm. per min.) and Erythrocyte Cell Volume (per cent)		MAXIMUM PER CENT DIFFERENCE IN SEDIMENTATION RATES	MAXIMUM PER CENT DIFFERENCE IN ERYTHROCYTE CELL VOLUME (PER CENT)
4.78 28.8%	4.82 28.7%	1	0.3
2.41 34.0%	2.50 34.0%	4	0.0
2.10 44.6%	2.08 44.4%	2	0.5
1.89 43.7%	1.84 43.7%	3	0.0
1.35 45.0%	1.34 44.9%	1	0.2
1.03 35.8%	1.03 35.8%	0	0.0
0.93 39.8%	0.93 39.7%	0	0.3
0.53 45.7%	0.53 45.5%	0	0.4
0.28 26.3%	0.26 26.4%	7	0.4
0.17 49.0%	0.17 49.1%	0	0.2
0.14 47.0%	0.14 47.2%	0	0.4
Average.....		2	0.3

per cent; for the erythrocyte cell volume the average difference was 0.3 per cent. For the experiments described below these figures were considered the expected error of the methods as employed here.

3. *Height of Blood Column.* It has been demonstrated by Fischel (26) and others (114, 121) that for samples of the same blood specimen suspended in tubes of the same internal diameter an increase in the height of the blood column produces acceleration of the sedimentation rate. Since the slope method of timing was not employed by these authors 5 experiments were performed in which samples of a pooled blood specimen were introduced into 3 Rourke-Ernstene tubes to column heights of 50, 75 and 100 mm. The data are shown in Table 3. Compared to the sedimentation rate at 50 mm. the average increase at 75 mm. was 23 per cent, and at 100 mm. was 33 per cent.

TABLE 3

Effect of Varying the Height of the Blood Column on the Uncorrected Sedimentation Rate of Samples of the Same Blood Specimen

UNCORRECTED SEDIMENTATION RATE			DIFFERENCE IN SEDIMENTATION RATE FOR COLUMN HEIGHTS OF	
Height of Blood Column			50 to 75 mm.	50 to 100 mm.
50 mm.	75 mm.	100 mm.		
<i>mm. per min.</i>	<i>mm. per min.</i>	<i>mm. per min.</i>	<i>per cent</i>	<i>per cent</i>
1.19	1.44	1.50	23	26
1.06	1.39	1.49	31	42
1.25	1.35	1.39	8	11
0.44	0.55	0.60	25	33
0.24	0.31	0.37	26	54
Average.....			23	33

The longest period of constant fall and the most gradual "packing" was observed for a column height of 200 mm. For a column height of 50 mm. the period of constant fall was relatively brief and the "packing" more abrupt, as shown by Bannick and coworkers (1).

4. *Internal Diameter of Sedimentation Tube.* There is agreement that the sedimentation rate is unaltered by tubes of internal diameters varying from 3 to 11 mm. but that tubes of an internal diameter of 2 mm. or less cause slowing and irregularities in the settling of the erythrocyte column (15, 112, 91, 121). Between the diameters of 2 mm. and 3 mm. the effect is not well defined, although Wintrobe and Landsberg (121) found no significant difference for tubes ranging in bore from 2.5 mm. to 11 mm. In the experiments reported below the influence of internal diameters of 2.5, 3, 4 and 5 mm. was in-

vestigated at a constant column height, either 50 or 100 mm, on samples from pooled blood specimens. The results of the experiments at a blood column height of 50 mm. are shown in Table 4. There was no significant difference between the sedimentation rates observed in tubes with internal diameters of 3, 4 and 5 mm, but for a diameter of 2.5 mm. the average slowing of the rate, 27 per cent, was significant. Similar results were obtained at a column height of 100 mm.

5. *Anticoagulants.* The anticoagulant employed may have a significant effect on both the settling velocity and the characteristics of the sedimentation curve. Solutions of sodium citrate are known

TABLE 4

Effect of Varying the Internal Diameter of the Sedimentation Tube on the Uncorrected Sedimentation Rate of Samples of the Same Blood Specimen Suspended at a Blood Column Height of 50 Millimeters

UNCORRECTED SEDIMENTATION RATE				DIFFERENCE IN SEDIMENTATION RATE FOR TUBE DIAMETERS OF	
Internal Diameter Sedimentation Tube				2.5 to 5.0 mm	3.0, 4.0, 5.0 mm
2.5 mm.	3.0 mm.	4.0 mm.	5.0 mm.		
mm per min	mm per min.	mm per min	mm per min	per cent	per cent
0.90	1.20	1.19	1.25	39	5
0.87	1.20	1.25	1.16	34	8
0.96	0.97	1.06	1.05	10	9
0.37	0.45	0.44	0.47	27	7
0.20	0.24	0.24	0.25	25	4
Average—				27	7

to cause a slower sedimentation rate than heparin and hirudin (95, 107, 112). Rourke and Plass (95) demonstrated in one observation on hemophilic blood that a small concentration of heparin, 20 mg per 100 cc. of mixture, caused no alteration of the sedimentation rate when compared to that of a sample containing no anticoagulant. The mixture of dry ammonium and potassium oxalate devised by Heller and Paul (50) has no effect, according to Wintrobe and Landsberg (121), on the sedimentation rate or erythrocyte cell volume when compared to the effect of heparin. There is disagreement concerning the influence of potassium oxalate (95, 121).

In the experiments reported below four anticoagulants, namely, heparin, the dry oxalate mixture of Heller and Paul, potassium oxalate

TABLE 5

Effect of Anticoagulants on the Uncorrected Sedimentation Rate and Erythrocyte Cell Volume Per Cent in Samples of Hemophilic Blood Compared to an Unaltered Sample

Uncorrected Sedimentation Rate (mm. per minute) and Erythrocyte Cell Volume (per cent)

NO ANTICOAGULANT	ANTICOAGULANT (MG. PER 100 CC. MIXTURE)								
	Heparin, 15 Per Cent					Dry Oxalate Mix- ture	Potas- sium Oxalate, 20 Per Cent	Sodium Citrate	
								Dry	3.8 Per Cent
	20 mg.	65 mg.	130 mg.	150 mg.	175 mg.	200 mg.	200 mg.	760 mg.	760 mg.
0.83 42.5%	0.83 43.1%	0.87 43.1%			0.96 42.9%		0.79 39.5%		
0.63 Clot	0.63 45.4%	0.75 44.9%		0.75 45.6%		0.61 45.6%			
0.53 45.5%		0.63 44.7%	0.66 44.6%				0.52 41.8%		
0.43 45.3%			0.54 44.3%				0.42 41.7%	0.33 38.4%	0.22 34.8%
0.31 46.3%				0.35 45.9%		0.32 45.9%			
Average Per Cent Difference of Un- corrected Sedimen- tation Rates Com- pared to Sample with No Antico- agulant	0	+14	+26	+16	+16	±3	-3	-23	-49
Average Per Cent Difference of Ery- throcyte Cell Vol- ume Per Cent Compared to Sam- ple with No Anti- coagulant	+1.4	+1.6	-2.1	-0.9	+0.9	-0.9	-8.5	-15	-23

and sodium citrate, were reinvestigated for their influence on both the sedimentation rate and on the erythrocyte cell volume percentage. To define the influence of any one anticoagulant the sedimentation

rates of samples of hemophilic blood were compared, both with and without an anticoagulant. The clotting time of the unaltered hemophilic blood specimens varied from 2 to 3 hours. The sedimentation rate and erythrocyte cell volume per cent produced by an anticoagulant thus could be standardized against the values observed on an *unaltered* sample of the same hemophilic blood specimen. Since the

TABLE 6

Effect of Anticoagulants on the Uncorrected Sedimentation Rate in Samples of Non hemophilic Blood Compared to the Effect of Heparin, 20 mg, and of Dry Oxalate Mixture, 200 mg, per 100 cc of Mixture

NUMBER OF OBSERVATIONS	RANGE OF UNCORRECTED SEDIMENTATION RATES	AVERAGE PER CENT DIFFERENCE BETWEEN UNCORRECTED SEDIMENTATION RATES		
		(a) Compared to Heparin, 20 mg per 100 cc Mixture		
		Anticoagulant (mg per 100 cc mixture)		
		Dry Oxalate Mixture	Heparin,* 15 Per Cent	
		200 mg	65 mg	130-175 mg
22	mm. per min 0.04-2.82	±6		
13	0.07-2.85		+15	+31
		(b) Compared to Dry Oxalate Mixture 200 mg per 100 cc Mixture		
		Heparin, 15 Per Cent	Potassium Oxalate, 20 Per Cent	Dry Oxalate Mixture
		65 mg	200 mg.	100 mg 300 mg
17	0.18-3.74	+16		
6	0.04-2.00		±4	
7	0.04-3.62			±3 ±3

* Five different lots of heparin, Hynson, Westcott and Dunning.

number of observations employing hemophilic blood was limited, additional observations were made on nonhemophilic blood specimens.

In both the hemophilic and nonhemophilic blood specimens three anticoagulants produced no significant alteration of the sedimentation rate, namely, *heparin*, 15 per cent solution, 20 mg.; *dry oxalate mixture*, 100 mg. to 300 mg.; *potassium oxalate*, 20 per cent solution, 200 mg. per 100 cc. of mixture. This is in agreement with the observations of Wintrobe and Landsberg (121). The data are shown in Tables 5 and 6. However, the larger concentrations of heparin

usually required to prevent coagulation, from 65 mg. to 150 mg., always produced acceleration of the sedimentation rate, which varied directly with the heparin concentration.

There was no significant alteration of the erythrocyte cell volume per cent by either heparin, from 20 mg. to 175 mg., or dry oxalate mixture, from 100 to 300 mg. per 100 cc. of mixture. However, potassium oxalate in 20 per cent solution, 200 mg. per 100 cc. of mixture, caused an average shrinkage of 8 per cent, with variations of from 7.3 to 9.4 per cent.

Sodium citrate solutions of the concentrations employed by Linzenmeier, Westergren and Cutler caused extreme retardation of the sedimentation rate, the decrease ranging from 33 to 46 per cent of the rate observed on a blood sample containing potassium oxalate, 200 mg. per 100 cc. of mixture. The least retardation, 23 per cent, occurred when sodium citrate was used as the dry salt. (Table 7). When the final concentration of sodium citrate in the mixture was constant, progressive dilution of the blood by less concentrated citrate solutions always produced progressive slowing of the sedimentation rate. This is in agreement with the observations of Fahraeus (24) and of Westergren (114). When, however, dilution was maintained constant, an increase in the final concentration of sodium citrate from 760 mg. to 1000 mg. per 100 cc. of blood caused no significant alteration of the sedimentation rate. The erythrocyte cell volume per cent was modified primarily by the dilution of citrate solutions but also by shrinkage, as indicated in Table 7.

6. Effect of Standing. When sodium citrate solutions were used, Fahraeus (24) and Westergren (114) found no change in the sedimentation rate after a blood sample had stood at room temperature for several hours. Using dry oxalate mixture, Wintrobe and Landsberg (121) found no change in sedimentation rate after the blood sample had stood for 4 hours at room temperature, or in the ice box at 9°C. with subsequent warming to room temperature. In the investigation reported here, with heparin, 130 mg. per 100 cc. of mixture, no change in the sedimentation rate occurred after 6 hours' standing at room temperature, as found by Gilligan (30). Standing for 24 hours always produced extreme retardation. Employing dry oxalate mixture, 200 mg. per 100 cc. of mixture, no change in the

sedimentation rate was observed after standing for 2 hours, but after 3 hours there was significant slowing in 3 of 7 experiments. After standing for 5 or 6 hours significant retardation occurred in 6 of 7 experiments, and after 24 hours the retardation was always extreme.

TABLE 7

Effect of Sodium Citrate on the Uncorrected Sedimentation Rate and Erythrocyte Cell Volume Per Cent Compared to Effect of Potassium Oxalate Solution and Heparin

Uncorrected Sedimentation Rate (mm. per minute) and Erythrocyte Cell Volume (per cent)

ANTICOAGULANT (MG. PER 100 CC. MIXTURE)

Heparin 15 Per Cent	Potassium Oxalate 20 Per Cent	Sodium Citrate					
		3 Per Cent	Dry	3 Per Cent	3.8 Per Cent	3 Per Cent	5 Per Cent
130 mg.	200 mg.	300 mg	760 mg	760 mg	760 mg.	1000 mg.	1000 mg
45.0%	1.27	0.85 40.7%		0.48 32.9%	0.61 34.5%		
44.3%*	0.42		0.33 38.4%		0.22 34.8%		
43.8%	0.27				0.16 34.8%	0.09 29.6%	0.15 32.3%
45.8%	0.13				0.08 35.8%	0.05 31.5%	0.08 31.0%
Average Per Cent Decrease in Un- corrected Sedimentation Rate Compared to Potassium Oxalate		33	23	62	46	64	41
Per Cent Dilution Produced by Sodium Citrate		10	0	25.3	20	33.3	20
Average Per Cent Decrease in Cell Volume Per Cent Compared to Heparin....		9.6	13	27	21.8	31.8	26

* Hemophilic blood.

7. *Temperature.* The accelerating effect on the sedimentation velocity of an increase in temperature has been observed by Westergren (114), DeCourcy (18), Gordon and Cohn (32), Rourke and Plass (95) and T'ang (107). This factor may be of practical importance only if there is wide variation in room temperature. Through the courtesy of Rourke and Plass, their sedimentation data, reported in

1929 (95), have been calculated by the slope method of timing. The sedimentation rates of 11 experiments showed an average acceleration of 15 per cent at 25°C. and of 87 per cent at 38°C., when compared to the rate observed at 20°C. For this reason all sedimentation rates were determined at a temperature controlled between 24° and 25°C. in the experiments reported here.

8. *Inclination of Tube.* The striking acceleration of the sedimentation rate caused by slight inclination of the tube from the vertical has been demonstrated frequently (71, 88, 121). Wintrobe and Landsberg (121) have shown that an inclination of only 2.3 per cent may cause an increase of 30 per cent in the settling velocity. This factor can be readily eliminated in practice by careful alignment of the sedimentation tube in a vertical position.

9. *Discussion and Summary.* It is apparent that variations in technique have a material influence on the sedimentation rate. First, the sedimentation rate of different blood samples can be properly compared by a measurement of the settling velocity during the period of constant fall. Since the internal diameter of a tube of 2.5 mm. or less causes retardation of the settling velocity, this factor and the factors of blood column height, tube inclination and temperature all may produce alteration of the sedimentation rate. The period of constant fall is of short duration and the "packing" is relatively abrupt for a column height of 50 mm. as compared to heights of 100 and 200 mm.

Dry oxalate mixture was the most satisfactory anticoagulant since it produced no alteration of either the sedimentation rate or erythrocyte cell volume when varied in concentration over a range of from 100 mg. to 300 mg. per 100 cc. of mixture. Although potassium oxalate caused no change in the sedimentation rate, the shrinking effect on the erythrocyte cell volume was significant and variable in amount and was materially influenced by variation in the concentration of from 100 mg. to 300 mg. per 100 cc. of mixture. Heparin in the small concentration of 20 mg. per 100 cc. of mixture was not an adequate anticoagulant. For concentrations of 65 mg. or higher, it caused acceleration of the sedimentation rate. Heparin is not well suited to a routine laboratory procedure because of its expense and

its susceptibility in solution to deterioration by moulds. The dry oxalate mixture of Heller and Paul is a satisfactory substitute, except that on standing at room temperature the sedimentation rate of blood samples containing dry oxalate mixture may show slowing in 3 hours, whereas with heparin the sedimentation rate is unaltered for 6 hours.

Sodium citrate solutions produce approximately a 40 per cent reduction of the sedimentation rate, primarily because of their dilution of the plasma. They also modify the hematocrit by dilution and by shrinkage.

The variable influence of technical factors in the five sedimentation methods discussed above prevents any satisfactory direct comparison or conversion of the units of one method to those of another. Modification of a technique may produce significant alteration of the sedimentation rate and of the characteristics of the sedimentation curve.

B. Erythrocyte Factors

Erythrocyte factors which concern the sedimentation rate in human blood specimens are the concentration of erythrocytes in suspension and the size of the individual cell. It has been clearly established that decrease in erythrocyte concentrations causes acceleration of the sedimentation rate (7, 10, 17, 18, 24, 35, 36, 37, 38, 41, 94, 96, 111, 114, 121). Nevertheless, the desirability of a correction for the effect of this variable factor is still debated in the literature (1, 17, 116) and, in the five sedimentation methods considered here, a correction method is an integral part of only two, those of Rourke and Ernstene (94) and of Wintrobe and Landsberg (121). Correction methods (35, 114) are available for but seldom applied to the Westergren technique (4, 91, 116). Variation in the average size of the individual erythrocyte also has an effect on the sedimentation rate (3). The quantitative influence of these erythrocyte factors has been reinvestigated, as described below.

1. Erythrocyte Concentration. a. Whole Blood. The influence of the erythrocyte concentration on the sedimentation rate is observed in the following experiment. As recorded in Figure 2, samples of the same blood specimen in which the erythrocyte cell volume was

adjusted to three different levels showed material differences in the sedimentation rates. The influence of this factor has been reduced to a graphic method for correction of the sedimentation rate to a normal erythrocyte concentration by several authors (35, 94, 114, 121). However, correction procedures have been criticized since they are based on data derived from *artificial* dilutions of whole blood with plasma and therefore may not be applicable to the erythrocyte variations occurring in disease. Accordingly, a test of the Rourke-Ernstene correction method was undertaken in clinical instances of anemia in which the following were known: erythrocyte cell volume per cent, mean corpuscular volume, mean corpuscular hemoglobin concentration, and the concentration of plasma fibrinogen, serum globulins and albumin.

On samples of whole blood from 21 subjects the effect of erythrocyte concentration was determined by adjusting the cell volume of a sample of each blood specimen to approximately the normal value of 45 per cent. On this adjusted sample and on a sample of the same blood specimen which was unmanipulated, the sedimentation rates were determined simultaneously. By this technique the actual difference between the two sedimentation rates could be obtained, the first rate observed after experimental *adjustment* of cell volume, the second determined by graphic "correction," employing the Rourke-Ernstene erythrocyte cell volume correction graph. As found by Rourke and Plass (95), control observations demonstrated that centrifugation and remixing of blood samples, the procedures required for adjustment of the erythrocyte cell volume, did not affect the sedimentation rate.

In observations on blood specimens from 17 of the 21 subjects the average difference between the two rates was 9 per cent, as shown in Table 8. The serum globulin concentrations for these specimens varied from normal levels to the abnormally elevated value of 5.25 grams per cent; the fibrinogen concentration varied from the normal value of 230 mg. to the abnormally elevated value of 660 mg. per 100 cc. of plasma.

However, in 14 observations on blood specimens from the other 4 subjects, excessive slowing of the sedimentation rate occurred at normal levels of the erythrocyte cell volume. Since these specimens were investigated by a modification of the above procedure, typical experiments are shown separately in Table 9. In the first 2 instances

extreme retardation of the sedimentation rate was observed on adjustment of the erythrocyte cell volume from an anemic level to a normal level. Conversely, in the second 2 instances retardation of the sedimentation rate was observed in the unmanipulated blood

TABLE 8

Comparison of the "Corrected" Sedimentation Rate of Whole Blood with the Uncorrected Sedimentation Rate of a Sample of the Same Blood Specimen in Which the Erythrocyte Volume Was Adjusted to 45 Per Cent

UNMANIPULATED BLOOD SAMPLE			ERYTHROCYTE CELL VOLUME ADJUSTED TO 45 PER CENT		PER CENT DIFFERENCE BETWEEN "CORRECTED" RATE (COLUMN 3) COMPARED TO UNCORRECTED RATE (COLUMN 4)	ERYTHROCYTE	
Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Sedimentation Rate "Corrected" to Cell Volume Per Cent of Adjusted Sample	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume		Mean Corpuscular Volume	Mean Corpuscular Hemoglobin Concentration
1	2	3	4	5	6	7	8
mm. per min.	per cent	per cent	mm. per min.	per cent		cu. microns	per cent
4.18	25.2	1.61	1.84	45.1	13	101	33
3.57	36.5	2.20	2.70	43.5	19	111	35
3.01	36.8	1.73	1.77	46.0	2	106	31
2.76	27.7	1.19	1.23	45.5	3	127	30
2.48	41.0	1.95	1.73	44.8	13	84	36
2.48	27.4	1.04	0.99	45.1	5	93	32
2.41	41.7	1.88	1.77	45.4	6	94	31
2.02	27.4	0.75	0.80	46.0	6	129	29
1.78	39.7	1.24	1.33	46.3	7	83	34
1.69	34.0	0.88	0.95	46.0	7	105	33
1.39	38.7	0.98	1.10	45.1	12	96	33
1.31	41.4	1.04	1.07	45.7	3	83	32
1.02	26.2	0.23	0.26	46.0	12	88	29
0.65	39.0	0.43	0.52	45.5	18	84	35
0.62	29.1	0.12	0.15	47.3	20	70	23
0.55	27.3	0.10	0.10	45.9	0	89	33
0.08	47.8	0.12	0.14	44.8	14	91	32
Average.					9		

sample at a normal hematocrit level and disappeared following adjustment to an anemic level. The fibrinogen concentrations for typical blood samples from these 4 subjects in the order shown in Table 9 were, respectively, 270 mg., 620 mg., 650 mg. and 450 mg. per

100 cc. of plasma; the serum globulin concentrations were, respectively, 8.2 grams, 3.2 grams, 3.1 grams and 3.0 grams per cent.

From these observations it was apparent that the sedimentation rate of blood samples with abnormally low levels of the erythrocyte cell volume was significantly greater than a sample of the same blood adjusted to an erythrocyte cell volume of 45 per cent (compare columns 1 and 4, Table 8). Except in certain instances of increased concentrations of serum globulins, the Rourke-Ernstene method cor-

TABLE 9

Examples of Excessive Retardation of the Sedimentation Rate of Whole Blood Samples with Normal or Slightly Elevated Erythrocyte Cell Volume Per Cent

UNMANIPULATED BLOOD SAMPLE			BLOOD SAMPLE WITH ADJUSTED ERYTHROCYTE CELL VOLUME PER CENT			PER CENT DIFFERENCE BETWEEN C.S.I. OF UNMANIPULATED SAMPLE (COLUMN 3) COMPARED TO C.S.I. OF ADJUSTED SAMPLE (COLUMN 6)	ERYTHROCYTE	
Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index		Mean Corpuscular Volume	Mean Corpuscular Hemoglobin Concentration
1	2	3	4	5	6	7	8	9
mm. per min.	per cent	mm. per min.	mm. per min.	per cent	mm. per min.		cu. microns	per cent
5.75	24.1	1.94	0.18	47.1	0.21	+825	84	37
4.66	29.5	1.94	0.37	48.2	0.47	+312	95	31
0.78	47.3	0.90	1.00	46.5	1.10	-18	103	33
			4.20	34.5	2.04	-56		
1.24	47.0	1.40	2.00	45.3	2.05	-32	99	32
			2.62	35.7	1.59	-12		
			3.92	25.3	1.55	-10		

rected satisfactorily for the influence of the erythrocyte concentration in actual instances of anemia associated with variation in the size of the cells. However, in some of the blood specimens with abnormally elevated concentrations of serum globulins, the excessive retardation of sedimentation rate observed at normal hematocrit levels produced large errors when the correction method was employed.

b. Defibrinated Blood. The sedimentation rate of defibrinated blood is subsequently discussed as related to constituents of serum and as related to the sedimentation rate of whole blood. The first step was

to define the influence of erythrocyte concentration on the sedimentation rate of defibrinated blood and to establish a correction method for this effect, as described in the following observations.

Blood specimens from 16 patients were defibrinated with glass beads, subdivided into four samples, and the erythrocyte cell volumes

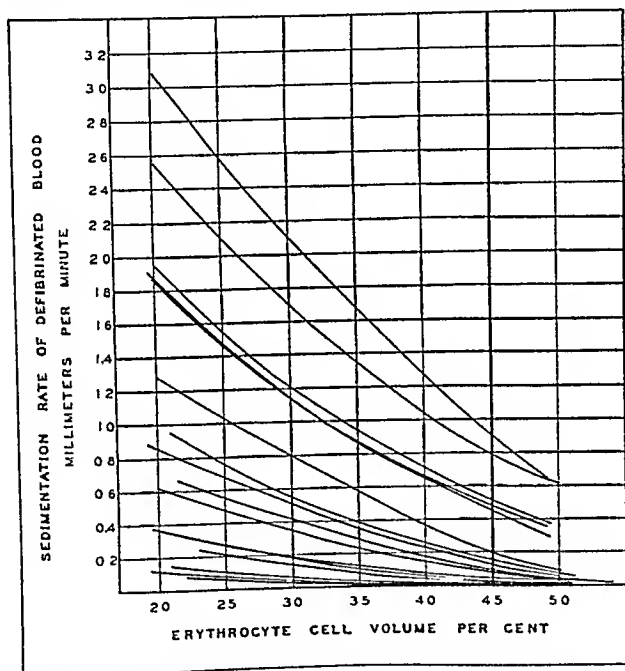


FIG. 3. UNCORRECTED SEDIMENTATION RATE OF DEFIBRINATED BLOOD SAMPLES AT DIFFERENT LEVELS OF THE ERYTHROCYTE CELL VOLUME PER CENT

adjusted to 20, 30, 40 and 50 per cent, respectively. The sedimentation rate and the erythrocyte cell volume per cent of each of the four samples were observed simultaneously. The results are shown in Figure 3. A graph for the correction of the sedimentation rate of defibrinated blood from the observed erythrocyte cell volume to 45

per cent was then constructed, Figure 4, by combining the curves obtained above with the curves determined by Rourke and Ernstene on whole blood (94). Therefore, the graph (Figure 4) represents an

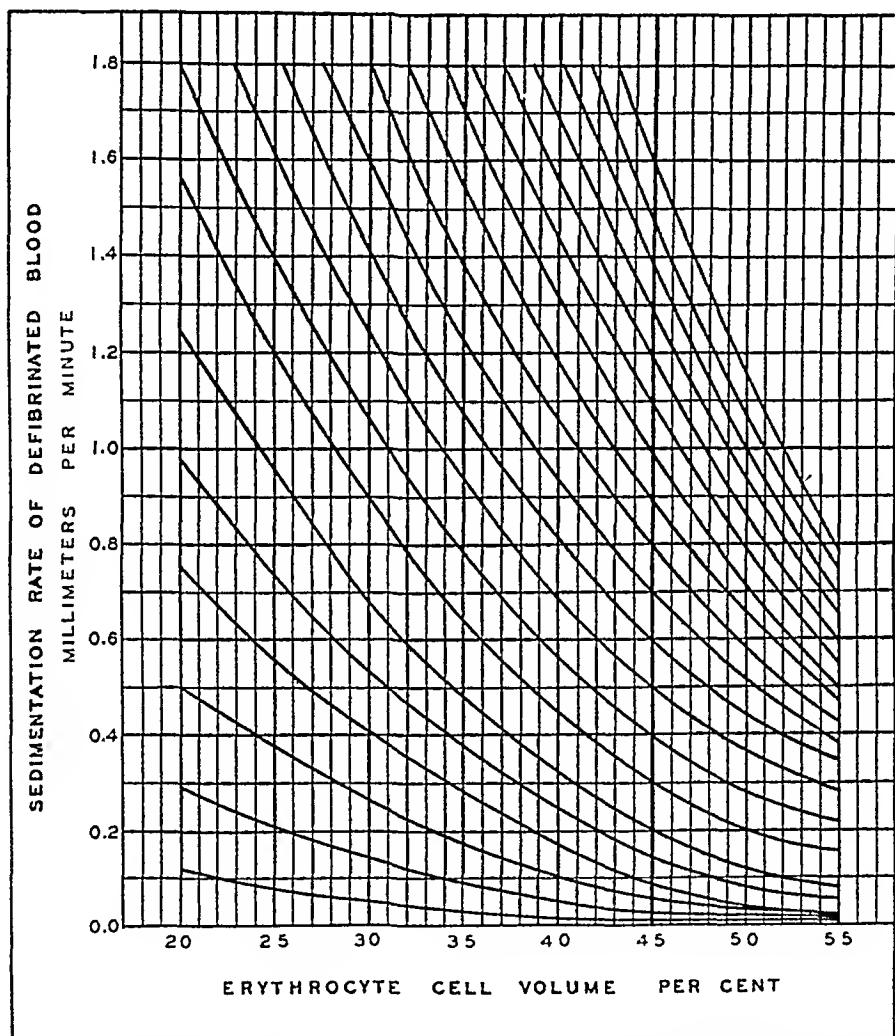


FIG. 4. GRAPH FOR CORRECTION OF THE SEDIMENTATION RATE OF DEFIBRINATED BLOOD FROM THE OBSERVED ERYTHROCYTE CELL VOLUME TO 45 PER CENT

For more rapid rates the correction chart of Rourke and Ernstene is employed

extension of the Rourke-Ernstene graph to include the slower sedimentation rates.

The validity of this correction method for defibrinated blood was then examined in 19 experiments on blood specimens from 16 individuals. The blood from 10 of these patients had not been studied

previously. The experiments were conducted as described for whole blood. The difference in per cent was obtained between, first, the sedimentation rate observed on a blood sample with the hematocrit

TABLE 10

Comparison of the "Corrected" Sedimentation Rate of Defibrinated Blood with the Uncorrected Sedimentation Rate of a Sample of the Same Blood Specimen with the Cell Volume Adjusted to 45 Per Cent

UNMANIPULATED BLOOD SAMPLE			ERYTHROCYTE CELL VOLUME ADJUSTED TO 45 PER CENT		PER CENT DIFFERENCE BETWEEN "CORRECTED" RATE (COLUMN 3) COMPARED TO UNCORRECTED RATE (COLUMN 4)	ERYTHROCYTE	
Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Sedimentation Rate 'Corrected' to Cell Volume Per Cent of Adjusted Sample	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume		Mean Corpuscular Volume	Mean Corpuscular Hemoglobin Concentration
1	2	3	4	5	6	7	8
mm per min	per cent	per cent	mm per min	per cent		cu microns	per cent
5 52	22 0	1 45	0 12	48 6	1100*	101	33
1 78	24 7	0 58	0 62	45 0	7	101	33
1 43	25 0	0 44	0 55	44 0	20	95	31
1 21	34 8	0 66	0 69	45 6	5	113	32
1 19	42 3	0 96	0 87	46 1	10	94	31
1 11	31 0	0 49	0 45	44 3	9	98	34
1 10	32 0	0 53	0 63	43 9	16	80	31
1 00	31 2	0 33	0 33	47 9	0	102	31
0 65	50 4	0 88	0 79	45 7	11	103	33
0 64	33 9	0 25	0 23	46 4	9	105	33
0 32	47 1	0 41	0 49	43 8	16	99	32
0 30	42 0	0 21	0 22	46 1	4	84	36
0 30	39 3	0 16	0 15	45 3	7	96	33
0 21	41 5	0 14	0 11	45 0	29	99	33
0 20	41 0	0 14	0 12	44 3	18	91	33
0 15	40 0	0 07	0 07	45 6	0	84	35
0 09	42 7	0 06	0 06	46 5	0	88	34
0 04	36 0	0 02	0 02	44 0	0	93	30
0 01	49 6	0 01	0 02	46 3	0	94	35
Average					8		

* Not included in average

adjusted to 45 per cent and, second, the sedimentation rate of an unmanipulated blood sample "corrected" to the hematocrit of the adjusted sample, employing the erythrocyte cell volume correction

tion rate, as observed by Bendien, Neuberg and Snapper (3). No correlation was found, however, between the sedimentation rate and the mean corpuscular hemoglobin concentration, as evidenced by the

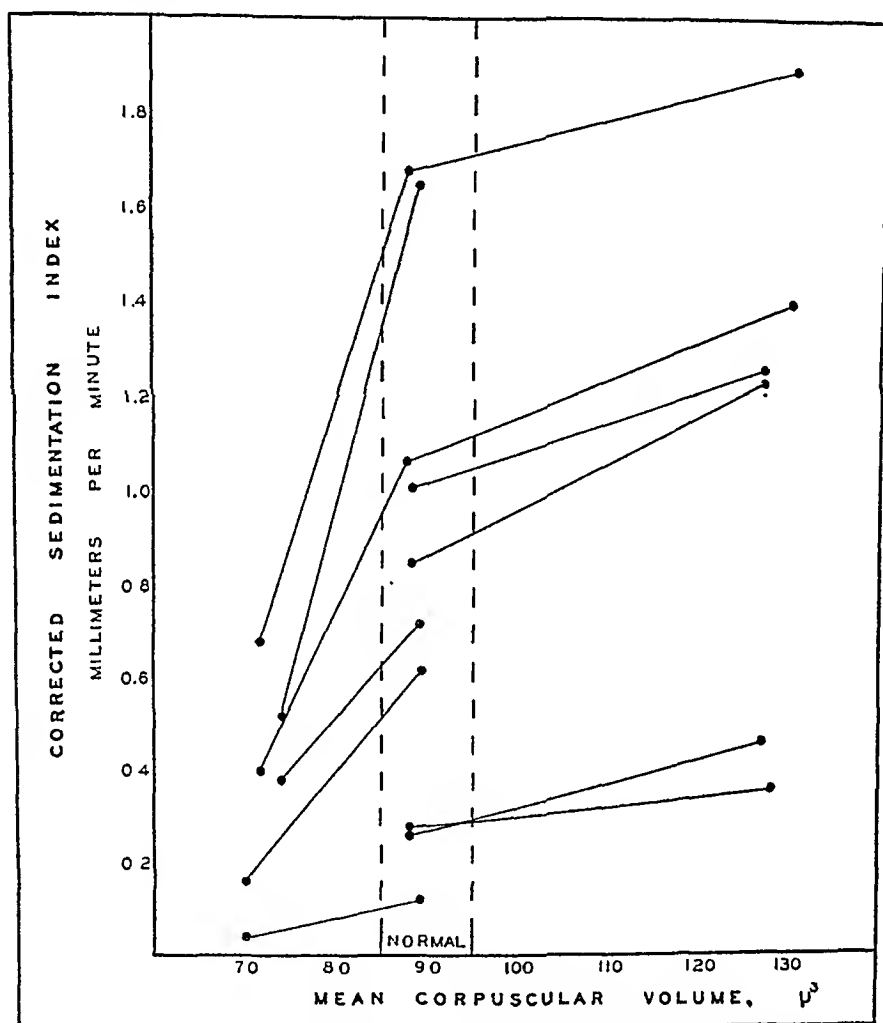


FIG. 5. CORRELATION BETWEEN THE CORRECTED SEDIMENTATION INDEX AND THE SIZE OF ERYTHROCYTES AS MEASURED BY THE MEAN CORPUSCULAR VOLUME

Lines connect observations in which samples of the same plasma were employed to suspend 2 or 3 different samples of erythrocytes.

data in Table 12. The erythrocyte cell volume correction method was satisfactory for obtaining the corrected sedimentation index for samples of the *same* erythrocyte size adjusted to different levels of the

erythrocyte cell volume per cent, as shown in Table 11 by comparing the data for tubes 1 to 5, 2 to 6, etc. Since the cell volume correction takes no account of differences in the *erythrocyte size*, however, it is subject to error due to this factor, as shown in Table 11 by comparing the data for tubes 1 to 2, 3 to 4, etc. In other words, the erythrocyte cell volume correction method corrects adequately in most instances for the *concentration* of erythrocytes, but cannot correct for the influence of microcytosis or macrocytosis.

TABLE 12

Comparison of the Sedimentation Rate of Macrocytic, Normocytic and Microcytic Erythrocytes Suspended in 2 Different Plasmas at Erythrocyte Cell Volume Adjusted to 45 Per Cent

PLASMA A					PLASMA B				
Tube Number	Erythrocytes in Suspension	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index	Tube Number	Erythrocytes in Suspension	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index
		mm per min	per cent	mm per min.			mm per min.	per cent	mm per min.
1	Microcytes	0.39	45.2	0.40	4	Microcytes	0.68	45.0	0.68
2	Normocytes	1.17	43.3	1.07	5	Normocytes	1.59	45.8	1.68
3	Macrocytes	1.45	44.4	1.40	6	Macrocytes	1.89	45.0	1.89

	Microcytes	Normocytes	Macrocytes
Red Blood Count	3.70 million	5.55 million	1.57 million
Hemoglobin. . .	50 per cent	105 per cent	39 per cent
Hematocrit	26.5 per cent	45.8 per cent	21.1 per cent
Mean Corpuscular Volume	72 cu. microns	88 cu microns	130 cu microns
Mean Corpuscular Hemoglobin Concentration.	29 per cent	34 per cent	29 per cent
Color Index.	0.68	0.95	1.2

changes in erythrocyte concentration except in certain instances of hyperglobulinemia. No method, however, based solely on the erythrocyte cell volume per cent can expect to correct for the effect of variation in erythrocyte size on the sedimentation rate. Decrease in erythrocyte size caused significant decrease of the settling velocity, which correlated with the mean corpuscular volume and color index, but not with the mean corpuscular hemoglobin concentration. No correction method is suggested for the effect of erythrocyte size. At normal hematocrit levels certain samples of whole blood with hyperglobulinemia showed excessive retardation of the sedimentation rate. The sedimentation rate of defibrinated blood showed excessive retardation in only one instance of extreme hyperglobulinemia.

C. Sedimentation Rate as a Measure of Fibrinogen, Serum Globulins and Lipoids

In general, it is established that there may be linear correlation between the sedimentation rate of whole blood and the concentration of plasma fibrinogen (4, 21, 24, 31, 37, 45, 46, 64, 82, 116). Fahraeus in 1921 (24) and others (64, 104) have demonstrated that washed erythrocytes suspended either in solutions of plasma fibrinogen or in plasmas of differing fibrinogen concentrations show degrees of aggregation and sedimentation rates which vary over a wide range and which may be roughly proportional to the fibrinogen level of the medium. For *defibrinated* blood, on the other hand, erythrocyte aggregation is usually minimal and the settling velocity extremely slow except in the occasional instances of elevated levels of the serum globulins, in which the sedimentation rate may be roughly proportional to the concentration of these globulins (4, 24). For the sedimentation rate of whole blood the apparent influence of plasma fibrinogen and of serum globulins may be additive (4, 24). With an increase in concentration of fibrinogen above normal levels, however, the sedimentation rate may show approximately eight times the acceleration observed for the same increase in concentration (in grams per cent) of serum globulins (64). The settling velocity of erythrocytes suspended in solutions of serum *albumin* is extremely slow (24, 64). Concerning *lipoids*, several investigators (40, 63, 66) have observed an acceleration

of the sedimentation rate with elevated levels of cholesterol and a decrease of the sedimentation rate with elevated levels of lecithin. Theorell (109) has observed marked slowing of the sedimentation rate from both cholesterol and lecithin with in vitro experiments. Clinically, Theorell (109) and coworkers (57, 116) found no correlation between the sedimentation rate and the lipid concentrations. However, no instances of gross lipemia were reported by these investigators.

The object of the following experiments is to examine the accuracy and specificity of the sedimentation test as an indirect quantitative method for the estimation of constituents of plasma and serum. The sedimentation rate of *whole blood* was investigated as a measure of plasma fibrinogen. The sedimentation rate of *defibrinated blood* was investigated as a measure of serum globulins and lipoids. For whole blood, the sedimentation rate was determined by the method of Rourke and Ernstene, using as anticoagulant dry oxalate mixture, 200 mg. per 100 cc. of blood, unless otherwise noted in the tables. All rates were corrected by the Rourke-Ernstene method to an erythrocyte cell volume of 45 per cent and reported as the corrected sedimentation index. For defibrinated blood the sedimentation rate was corrected by the method described here to an erythrocyte volume of 45 per cent and reported as the corrected defibrinated sedimentation rate.

1. *Whole Blood Sedimentation Rate as a Measure of Fibrinogen.* The sedimentation rate of whole blood was investigated in normal subjects, and in patients whose blood samples showed abnormal concentrations of fibrinogen, serum globulins and lipoids. The relationship between the sedimentation rate of whole blood and of defibrinated blood is discussed under the following four classifications.

a. *Normal Blood.* In 20 experiments on blood samples from 18 normal subjects (Table 13) the range for the corrected sedimentation index (whole blood) was from 0.04 to 0.43 mm. per minute. The upper limit of normal was approximately 0.4 mm. per minute, which is in agreement with the observations of D. Rourke Gilligan (30), using heparin as anticoagulant.

For *defibrinated blood*, in 33 experiments on 29 normal subjects (Table 14) the corrected defibrinated sedimentation rate was ex-

tremely slow, about one-tenth the velocity for whole blood, with a range of sedimentation rates of from 0.01 to 0.03 mm. per minute and an average rate of 0.018 mm. per minute.

TABLE 13

Plasma Fibrinogen Concentration and Sedimentation Rate Determined by Two Methods on Whole Blood from Normal Subjects

FIBRINOGEN	ROURKE-ERNSTENE METHOD			WINTROBE-LANDSBERG METHOD	
	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index	Uncorrected Sedimentation Rate	Corrected Sedimentation Rate
Females					
mg. per 100 cc. plasma	mm. per min.	per cent	mm. per min.	mm. in 1 hour	mm. in 1 hour
290	0.56	41.1	0.43	20.6	19.0
290	0.34	43.2	0.29	12.9	14.0
280	0.40	43.7	0.36	14.6	16.5
280	0.10	47.9	0.14	2.1	8.0
280	0.36	40.5	0.23	14.0	12.0
270					
260	0.19	41.2	0.14	6.4	5.5
260	0.30	40.8	0.19	11.2	10.0
240	0.29	42.7	0.23	10.9	12.0
Males					
270	0.40	45.2	0.40	15.0	13.0
270	0.22	39.5	0.12	9.2	1.5
260	0.36	46.4	0.40	11.8	11.0
250	0.15	44.3	0.13	4.1	2.0
240					
230					
230	0.05	50.7	0.11	0.3	3.5
230	0.06	46.7	0.09	1.3	1.0
230	0.07	45.9	0.09	2.0	1.0
220	0.07	45.8	0.09	2.0	1.0
220	0.10	45.8	0.13	2.8	2.0
210	0.06	45.4	0.07	2.0	1.0
210	0.18	40.9	0.12	6.3	0.5
190	0.03	45.4	0.04	0.8	0
250 Average					

b. Abnormal Elevation of Fibrinogen. The sedimentation rate was investigated for whole blood and for defibrinated blood on samples

from 17 subjects with the following abnormalities: acute and chronic infections, bleeding peptic ulcer, intravenous injection of typhoid vaccine, anemia from hemorrhage and nephritis. The data are shown in Table 15. The blood specimens from these patients showed elevated concentrations of fibrinogen but normal levels of serum globulins and of erythrocyte mean corpuscular volume (47).

TABLE 14
Sedimentation Rate of Defibrinated Blood from Normal Subjects

FEMALES			MALES		
Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Rate	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Rate
<i>mm. per min.</i>	<i>per cent</i>	<i>mm. per min.</i>	<i>mm. per min.</i>	<i>per cent</i>	<i>mm. per min.</i>
0.034	45.5	0.04	0.10	43.4	0.08
0.039	42.1	0.03	0.036	43.4	0.03
0.044	38.9	0.02	0.022	48.8	0.03
0.036	41.8	0.02	0.015	45.6	0.02
0.032	39.7	0.02	0.016	42.1	0.01
0.025	40.0	0.02	0.014	44.7	0.01
0.019	45.0	0.02	0.011	50.5	0.01
0.019	43.3	0.02	0.01	49.0	0.01
0.015	45.0	0.02	0.01	47.3	0.01
0.021	40.9	0.01	0.01	44.7	0.01
0.017	43.2	0.01	0.009	48.9	0.01
0.017	42.6	0.01	0.009	48.4	0.01
0.016	42.0	0.01	0.008	45.7	0.01
0.011	43.0	0.01	0.008	45.2	0.01
0.011	41.0	0.01	0.007	48.8	0.01
0.01	43.0	0.01	0.007	48.8	0.01
			0.004	47.3	0.01
Average....		0.018			0.018

A linear correlation between the corrected sedimentation index and the plasma fibrinogen is apparent from Figure 6, in which has been plotted no more than 4 observations from any one case. This corroborates the observations of Gilligan and Ernstone (31). Treated statistically, the coefficient of correlation between the plasma fibrinogen concentration and the corrected sedimentation index for the 52 determinations shown in Table 15 was $r = 0.94 \pm 0.01$.

Although the coefficient of correlation is high, it does not express

TABLE 15

Plasma Protein, Erythrocyte and Sedimentation Rate Data on Blood Specimens Showing Variable Concentrations of Plasma Fibrinogen but Normal Levels of Serum Globulins and of Erythrocyte Mean Corpuscular Volume

CASE NUMBER	DATE	BLOOD CHEMISTRY						ERYTHROCYTES		SEDIMENTATION RATES										DIAGNOSIS	
		Plasma Proteins					Fibrinogen	Mean Corpuscular Volume	Mean Corpuscular Hemoglobin Concentration	Rourke-Ernstene Method Whole Blood				Defibrinated Blood				Wintrobe Method Whole Blood			Westergren Method Whole Blood
		Serum Globulin	Albumin	Total Protein	Non Protein	Cholesterol				Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Rate	Uncorrected Sedimentation Rate	Corrected Sedimentation Rate	Uncorrected Sedimentation Rate	Corrected Sedimentation Rate		
		mg. per 100 cc.	gm. per cent	gm. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.		cu. mm.	per cent	per cent	mm. per min.	per cent	mm. per min.	mm. per min.	per cent	mm. per min.	mm. per min.	mm. in 1 hr.	mm. in 1 hr.	mm. in 1 hr.	mm. in 1 hr.
1	5/18/35	440						93	30	0.79	40.0	0.58									
	5/20/35	620								2.81	35.3	1.66									
	5/20/35	620	1.26	2.68	4.56	20				3.03	35.2	1.79	0.04	36.0	0.02						
	5/21/35	730								2.96	37.8	1.93									
																					Lobar Pneumonia (Autopsy)
2	4/16/35	530								1.24	46.4	1.36						34.3	33.0		
	4/17/35	570	1.95	3.64	6.15	36		91	33	1.67	44.0	1.57	0.09	44.2	0.08			44.0	38.5		
	4/18/35	630								1.62	46.1	1.75						40.0	38.4		
	4/24/35	460	2.02	3.33	5.90	29				1.22	41.3	1.0	0.06	43.3	0.04			43.9	33.5		
	4/30/35	550								1.70	38.9	1.24	0.16	40.4	0.08						
																					Acute Upper Respiratory Infection

3	10/10/35	550	2.65	3.5	6.72	13		99	32	1.30	44.5	1.27	0.02	46.3	0.03	38.3	34.0	31.0	Chronic Gonocoe- cal Prostatitis, Urethritis, Ar- thritis
	10/21/35	540								0.85	45.4	0.88				27.5	25.0	25.2	
	10/26/35	570								1.09	48.0	1.30				30.1	31.5		
	10/31/35	490	2.33	3.36	6.17	18		93	33	0.87	43.3	0.78	0.03	43.0	0.02	32.0	26.5	25.7	
	11/16/35	430								0.66	47.1	0.77				20.0	20.0	13.2	
	11/20/35	360								0.42	43.6	0.39				16.2	11.0		
4	1/16/35	510	2.21	3.45	6.17	29				2.02	42.8	1.77	0.27	45.2	0.27	43.0	36.0	69.0	Lobar Pneumonia
	1/18/35	480	2.57	3.24	6.32	29	83	34		1.81	40.7	1.44	0.21	46.0	0.22			58.0	
5	7/18/35	370	1.96	4.47	6.80	30							0.05	48.5	0.09				Rheumatic Fever
	7/22/35	460					93			1.24	44.0	1.18				42.8	37.5		
	7/23/35	430								1.19	42.2	1.01				43.7	35.5		
6	1/ 4/36	440	2.39	4.24	7.08	30		96	28	1.99	24.5	0.68	0.30	24.9	0.04			60.0	Peptic Ulcer, Bleeding
7	1/14/35	430	1.86	3.06	5.35	34		83	32	1.31*	41.4	1.08	0.13	45.3	0.13	44.0	34.0	44.0	Lobar Pneumonia
8	10/12/35	300							34	0.50	49.2	0.65				13.9	16.5	6.8	Intravenous Injec- tion Typhoid Vaccine
	10/17/35	310	2.16	3.94	6.42	27		95	34	0.41	49.2	0.53	0.01	49.6	0.02	12.1	15.0	4.2	
	10/18/35	310						94	35	0.40	49.7	0.53				12.0	15.0	4.0	
	10/24/35	290						90	35	0.27	47.2	0.33				10.7	11.0	4.0	
	11/ 6/35	420	2.04	4.11	6.60	34		92	33	0.74	46.9	0.83	0.022	48.1	0.03	28.0	28.0	16.0	
	1/14/36	280	2.24	3.81	6.32	31		93	36	0.32	44.7	0.32	0.019	45.1	0.02	11.9	9.5		
9	7/ 6/35	420	2.40	3.46	6.27	27	144	89	33	1.31	43.6	1.21	0.25	41.3*	0.17	43.0	37.0		Lobar Pneumonia
10	4/ 9/35	230						91	32	0.07	46.9	0.10				1.0	1.0		Normal
	2/11/36	220								0.07	45.8	0.09				2.0	1.0		Acute Upper Res-
	2/18/36	400								0.36	46.0	0.39				14.2	13.0		piratory Infec- tion
	3/ 9/36	280	1.62	3.88	5.78	23				0.10	46.4	0.13	0.004	47.3	0.01	2.9	2.0		

* Heparin as anticoagulant, 130 mg. per 100 cc. blood.

CASE NUMBER	DATE	BLOOD CHEMISTRY						ERYTHROCYTES		SEDIMENTATION RATES								DIAGNOSIS	
		Plasma Proteins					Cholesterol	Mean Corpuscular Volume	Mean Corpuscular Hemoglobin	Rourke-Ernstene Method Whole Blood			Defibrinated Blood			Wintrobe Landsberg Method Whole Blood			Westergren Method Whole Blood
		Fibrinogen	Serum Globulin	Albumin	Total Protein	Non Protein				Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Rate	Uncorrected Sedimentation Rate	Corrected Sedimentation Rate		
		mg. per 100 cc.	gm. per cent	gm. per cent	gm. per cent	mg. per 100 cc.	mg. per 100 cc.	cu. microns	per cent		mm. per min.	per cent	mm. per min.	mm. per min.	per cent	mm. per min.	mm. per 1 hr.	mm. per 1 hr.	mm. per 1 hr.
11	10/11/35	300									0.80	41.3	0.63			28.6	27.0		Gonococcal Pelvic Inflammation
	10/12/35	320									0.80	43.2	0.71			31.4	33.0		
	10/14/35	390	1.98	5.03	7.41	19	97	98	33		1.17	41.0	0.93	0.21	41.5	0.14	42.4	40.5	
12	4/15/35	270						90	34		0.40	45.2	0.40			15.0	13.0		Intravenous Injection Typhoid Vaccine
	4/26/35	380	1.68	3.91	5.96	26		93	35		0.82	42.9	0.72	0.04	43.8	0.03	34.8	28.3	
	4/27/35	380									0.86	44.7	0.85			31.2	27.5		
	5/ 1/35	310									0.51	45.7	0.58			20.2	18.6		
13	12/ 6/35	370						97	32		0.45	40.0	0.31			18.3	9.5	10.8	Peptic Ulcer, Bleeding; Hypertension
	12/10/35	300	1.77	3.67	5.75	25		91	35		0.35	38.2	0.18	0.18	39.0	0.08	18.2	8.0	
	12/16/35	280						84	34		0.38	40.4	0.25					7.2	
14	4/19/35	280	2.28	3.44	6.01	24					0.40	42.1	0.32	0.02	42.4	0.02			Atrophic Arthritis, Intravenous Injection Typhoid Vaccine
	4/23/35	350									0.58	41.7	0.46			13.6	13.0		
	4/25/35	300	1.88	3.64	5.82	26		87	34		0.65	40.0	0.48	0.04	40.5	0.02	18.4	17.5	
	4/27/35	300									0.50	41.2	0.38						

15	4/11/35	340							0.94	41.2	0.74						35.6	34.0	Pleurisy, Tuberculous
	5/ 1/35	340							0.82	41.0	0.62								
	5/17/35	280	1.79	4.18	6.25	21	88	34	0.65	42.2	0.55	0.09	42.7	0.07					
	7/12/35	230	1.57	4.48	6.30	22			0.38	41.9	0.29	0.09	38.0*	0.04	15.4	15.0			
16	3/13/36	270	1.54	3.90	5.71	16	87	36	0.22	39.5	0.12	0.02	40.1	0.01	9.2	1.5			Intravenous Injection Typhoid Vaccine
	3/25/36	250	2.02	4.43	6.70	20			0.15	44.3	0.13	0.02	45.6	0.02	4.1	2.0			
	3/31/36	280	1.80	4.03	6.11	22			0.22	41.9	0.15	0.02	42.1	0.01	8.7	3.5			
17	3/ 5/36	280	1.60	2.66	4.56	19	189	83	0.86	29.8	0.27	0.10	30.1	0.02	33.1	14.0			Chronic Active Glomerulonephritis
18	12/12/35	270	1.75	4.23	6.25	24	88	29	0.26*	46.0	0.27								Normal
19	2/ 4/36	270	2.08	4.25	6.60	22	91	33	0.31	49.6	0.50	0.10	43.4	0.08	8.8	12.0			Normal
20	1/24/36	240	1.73	4.12	6.10	25	89	33	0.10	45.9	0.13				3.0	2.0			Normal

TABLE 15A

Plasma Protein, Erythrocyte and Sedimentation Rate Data on Blood Specimens Showing Variable Levels of Erythrocyte Mean Corpuscular Volume but Normal Concentrations of Plasma Fibrinogen and of Serum Globulins

1	12/17/35	330	2.17	3.22	5.72	22		104	29	1.07	31.9	0.47	0.16	31.9	0.04	46.0	20.0	36.5	Scurvy
2	1/ 9/36	260	2.11	3.05	5.42	24		100	30	0.77	40.7	0.58	0.05	41.9	0.03	30.9	29.0	17.5	Pernicious Anemia
3	12/ 6/35	190	2.66	3.75	6.20	16		78	28	0.40	23.5	0.05				18.6	0	8.2	Hypochromic Anemia

the arithmetic error of estimating the fibrinogen concentration from the corrected sedimentation index. The average error and the extreme

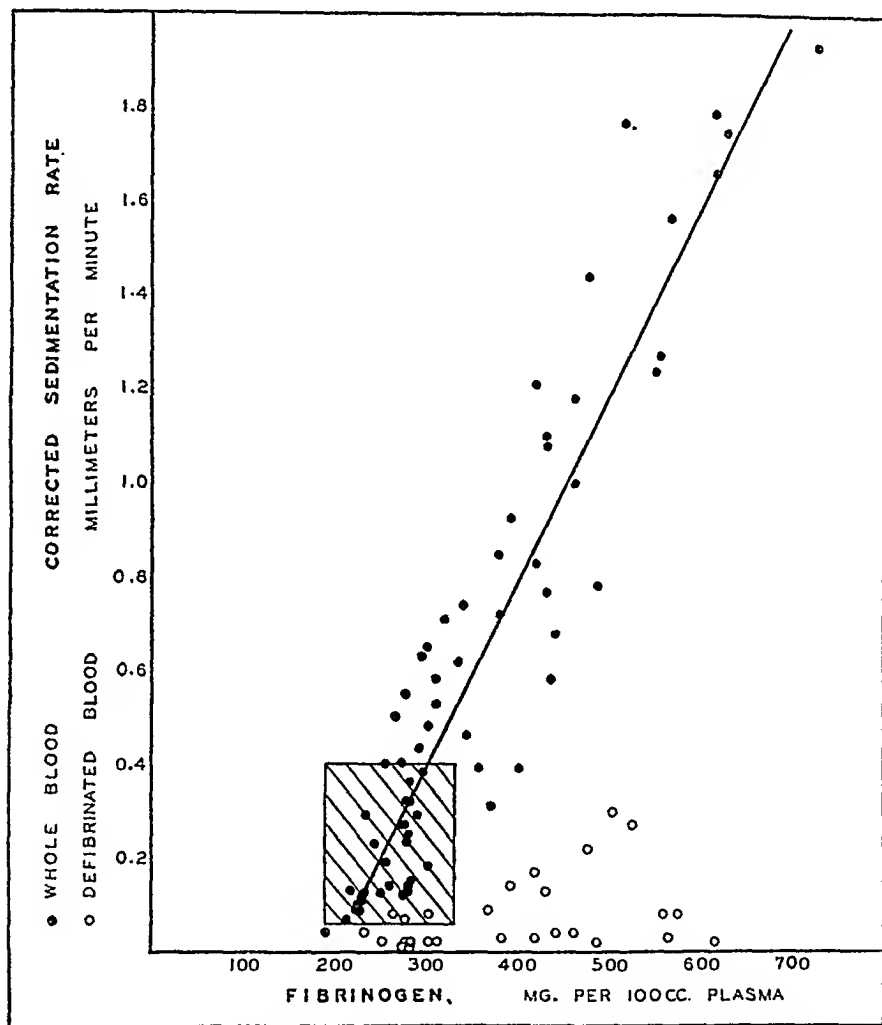


FIG. 6. CORRELATION BETWEEN PLASMA FIBRINOGEN AND THE CORRECTED SEDIMENTATION RATES OBSERVED ON WHOLE BLOOD (ROURKE-ERNSTENE METHOD) AND DEFIBRINATED BLOOD IN INSTANCES OF VARIABLE CONCENTRATIONS OF FIBRINOGEN BUT NORMAL CONCENTRATIONS OF SERUM GLOBULINS AND NORMAL MEAN CORPUSCULAR VOLUME.

The sedimentation rate of defibrinated blood is plotted beneath the corresponding whole blood rate in each instance. The diagonal line is the fibrinogen-sedimentation correlation line observed by Gilligan and Ernstene. The cross-hatched area indicates the normal range.

error, therefore, were determined by comparing the fibrinogen, as calculated from the corrected sedimentation index, with the fibrinogen

determined chemically. A total of 187 observations on 100 individuals were examined, including the data from Tables 13 and 15 together with 107 determinations already reported by Gilligan and Ernstene (31) and restudied here through the courtesy of these authors. The Gilligan and Ernstene determinations may be included properly in the above group showing abnormal elevation of fibrinogen with normal levels of serum globulins and of erythrocyte size.

The formula employed for calculating the fibrinogen from the corrected sedimentation index was suggested by Professor Edwin B. Wilson of the Harvard School of Public Health:

$$\text{Calculated } y = \left(\frac{\sum [(y - \bar{y})(x - \bar{x})]}{\sum (x - \bar{x})^2} \cdot (x - \bar{x}) \right) + \bar{y} \quad (1)$$

where (calculated y) is the *calculated* fibrinogen concentration in milligrams per 100 cc. of plasma; (y) the *observed* fibrinogen in milligrams per 100 cc. of plasma; (\bar{y}) the mean observed fibrinogen concentration; (x) the corrected sedimentation index in millimeters per minute and (\bar{x}) the mean value; (Σ) has its usual connotation, the sum of all observations. For the above data the values of the constants were:

$$\text{Calculated } y = 258 (x - 0.7) + 393 \quad (2)$$

The fibrinogen concentration was then calculated from formula (2) for each determination of the corrected sedimentation index and compared to the fibrinogen level determined chemically, the difference or error being expressed in per cent. For the whole series of 187 determinations the average arithmetic error of calculating fibrinogen from the sedimentation rate was 10 per cent, with extremes of -29 and +41 per cent. The errors of calculating fibrinogen at five different levels of the corrected sedimentation index are shown in Table 16.

The corrected *defibrinated* sedimentation rates determined on blood samples from the 20 subjects listed in Table 15 were extremely slow compared to the rates for whole blood. However, in this series the range of velocities was greater than the range observed in normal subjects, varying from 0.01 to 0.27 mm. per minute with an average of 0.06 mm. per minute for 29 determinations.

A definite correlation existed between the level of the corrected defibrinated sedimentation rate and the degree of accuracy with which

fibrinogen was calculated from the corrected sedimentation index. Thus in Cases 4, 5, 7, 9, 11, 15 and 19, Table 15, the calculated fibrinogen was disproportionately elevated above the value determined chemically by the following percentages, +26, +14, +14, +26, +32, +15 and +26, respectively. In each instance the corrected defibrinated sedimentation rate was elevated above normal, 0.27, 0.09, 0.13, 0.17, 0.14, 0.07 and 0.08 mm. per minute, respectively.

c. Abnormal Elevation of Serum Globulins and Variable Fibrinogen. The sedimentation rate was investigated for whole blood and de-

TABLE 16

Arithmetic Error of Calculating the Plasma Fibrinogen Concentration from the Corrected Sedimentation Index of Rourke and Ernstene in Blood Samples with Normal Levels of Serum Globulins and of Mean Corpuscular Volume

Fibrinogen values calculated from formula (2):

$$\text{Calculated } y = 258 (x - 0.7) + 393$$

where (calculated y) is the calculated fibrinogen concentration in milligrams per 100 cc. of plasma; (x) is the corrected sedimentation index in millimeters per minute.

RANGE CORRECTED SEDIMENTATION INDEX	NUMBER OF DETERMINATIONS	ERROR IN CALCULATING FIBRINOGEN CONCENTRATION FROM CORRECTED SEDIMENTATION INDEX	
		Range	Average
<i>mm. per min.</i>		<i>per cent</i>	<i>per cent</i>
0.04 to 0.34	73	-22 to +26	8
0.35 to 0.58	28	-22 to +29	12
0.60 to 0.85	28	-16 to +27	10
0.86 to 1.44	29	-26 to +36	12
1.47 to 2.35	29	-29 to +41	12
Total.....	187	-29 to +41	10

fibrinated blood in samples from 13 subjects with the following abnormalities: liver cirrhosis, primary carcinoma of the liver, toxic hepatitis following arsphenamine, chronic gonococcal arthritis, congestive heart failure, pernicious anemia, aleukemic leukemia and multiple myelomata. The data are shown in Table 17. The blood specimens from these subjects showed elevated concentrations of serum globulins and variable values for plasma fibrinogen. It was impossible to obtain this series of patients without including subjects with moderate macrocytosis of erythrocytes, Cases 2, 3, 5, 7, 9, 11

and 13, and one with advanced macrocytosis, Case 8. (It was obvious, however, that the alterations of the sedimentation rate described below were greater than could occur from macrocytosis alone.) No investigation was made of the influence of the various fractions composing the serum globulins, i.e., euglobulin, pseudoglobulin I, II. The Takata-Ara reaction was positive for all 13 patients.

In Figure 7 the fibrinogen concentration is plotted against the corrected sedimentation rates of both whole blood and defibrinated blood. For the 26 determinations on 13 subjects a vertical line connects the whole blood and defibrinated blood sedimentation rates of each sample. The failure of the corrected sedimentation index to show a linear correlation with fibrinogen is apparent from the figure and from the coefficient of correlation, which was $r = 0.57 \pm 0.09$. The calculated fibrinogen, employing formula (2), compared to the value determined chemically showed an average error of 64 per cent and extremes of -3 to +200 per cent.

The range of variation of the corrected defibrinated sedimentation rate was wide, varying from 0.11 to 1.83 mm. per minute. Elevation of the defibrinated rate was associated with an acceleration of the whole blood sedimentation rate which was out of proportion to the fibrinogen concentration in most instances. For normal or moderately elevated fibrinogen levels, as seen in Cases 1, 2, 3, 5, 6, 8 and 10 in Table 17, a rapid defibrinated rate was associated with extreme acceleration of the whole blood rate. However, when the fibrinogen level was elevated above 500 mg., Cases 4, 7, 9, 11 and 12, a rapid defibrinated rate was associated with slight or no acceleration of the whole blood rate, possibly because the latter was incapable of further increase in settling velocity.

d. Abnormal Elevation of Lipoids, Variable Fibrinogen, Normal Serum Globulins. The sedimentation rate was investigated for whole blood and for defibrinated blood in samples from 8 subjects with the following abnormalities: nephrotic syndrome associated with chronic nephritis, diabetes mellitus and carotinemia, as shown in Table 18. The blood specimens from these subjects showed elevated concentrations of lipoids as evident from the high plasma cholesterol levels, variable values for plasma fibrinogen and the erythrocyte mean

TABLE 17
Plasma Protein, Erythrocyte and Sedimentation Rate Data on Blood Specimens Showing Abnormally Elevated Concentrations of Serum Globulins and Variable Levels of Plasma Fibrinogen and of Erythrocyte Mean Corpuscular Volume

CASE NUMBER	DATE	BLOOD CHEMISTRY						ERYTHROCYTES		SEDIMENTATION RATES						CLINICAL DIAGNOSIS	
		Plasma Proteins						Mean Corpuscular Volume	Mean Globin Concentration	Rourke-Ernstene Method Whole Blood			Defibrinated Blood				
		Serum Globulins	Fibrinogen	Albumin	Total Protein	Non Protein Nitrogen	Cholesterol			Takata Ara Reaction	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume	Corrected Sedimentation Index	Uncorrected Sedimentation Rate	Erythrocyte Cell Volume		Corrected Sedimentation Rate
gm. per cent	mg. per 100 cc.	gm. per cent	gm. per cent	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	%	per cent	mm. per min.	per cent	mm. per min.	mm. per min.	per cent	mm. per min.			
1	1/ 4/35	7.75	270	3.18	11.2	76	4+	84	37	5.75*	24.1	1.94	5.52	22.0	1.83	Multiple Myeloma†	
	1/ 9/35	8.20	240	2.86	11.3	67	4+			6.49*	22.8	1.99					
2	11/19/35	5.25	220	1.76	7.26	58	4+	105	33	1.69	34.0	0.94	0.637	33.9	0.27	Syphilis, Tertiary† Hepatitis, Arspenamine	
	11/20/35		240							1.71	33.4	0.93					
3	12/19/34		290				4+	106	31	2.85*	36.0	1.75				Liver Cirrhosis, Biliary	
	12/21/34	5.14	310	2.30	7.75	25		106	31	3.01*	36.8	1.88	1.10	46.5†	1.21		
4	6/10/35	3.28	740	2.51	6.53	32	4+			5.10	29.3	1.97	1.11	31.0	0.46	Liver Carcinoma, Primary†	
	6/19/35	3.32	690	2.52	6.54	31		98	34	4.67	29.8	1.94	1.06	31.1	0.43		
	7/ 2/35	3.21	620	2.24	6.07	28				4.87	29.9	1.96	1.09	30.6	0.43		
	9/24/35	3.73	720	2.28	6.74	32		95	31	6.53	24.0	2.04	1.43	25.0	0.41		

5	3/ 5/36	3.59	280	3.14	7.00	30	237	4+	101	33	4.17	24.9	1.60	1.78	24.7	0.57	Liver Cirrhosis, Thyroidectomy Myxedema, Post-operative
6	9/14/35	3.56	240	1.18	4.97	23		4+	91	31	2.41	41.7	1.94	1.19	42.3	1.03	Liver Cirrhosis, Alcoholict
7	11/15/35	3.51	520	4.07	8.10	21		+	99	32	1.41	45.1	1.42	0.14	48.3	0.20	Chronic Gonococcal Urethri- tis, Arthritis
	11/18/35	3.26	460	3.83	7.55	22		+	100	33	1.48	43.7	1.37	0.25	38.0†	0.12	
	12/11/35	2.82	490	3.82	7.12	21		±			1.56	43.1	1.40	0.18	43.9	0.16	
	1/14/36	3.02	450	3.96	7.42	23					1.02	42.9	0.91	0.08	44.1	0.07	
8	12/12/34	3.18	350	3.37	6.90	42			127	30	2.76*	27.7	1.23				Pernicious Anemia
	12/18/31	2.92	290	3.45	6.66	35			129	29	2.02*	27.4	0.79	0.10	45.7	0.11	
9	9/30/35	3.13	710	3.39	7.23	14		4+	103	33	2.40	44.5†	2.29	0.79	45.7†	0.82	Rheumatic Heart Disease, Congestive Failure, Liver Cirrhosis
	10/ 4/35		650								4.20	34.5†	2.04				
10	12/ 7/31	3.12	210	2.33	5.69	35		4+	93	32	2.48*	27.4	1.04	0.34	45.6†	0.35	Liver Cirrhosis
11	2/25/36	3.11	550	3.12	6.78	15	170	4+	111	35	3.57	36.5	2.00	0.96	37.7	0.62	Multiple Myeloma†
12	1/28/36	2.91	590	3.50	7.03	24		4+	94	34	3.27	40.8	2.15	0.66	41.8	0.55	Alcukemic Leukemia†
	2/ 5/36	3.01	640	3.52	7.20	27		4+	89	35	3.90	39.8	2.30	0.71	40.7	0.55	
13	10/ 1/35	2.98	450	3.24	6.68	22			99	32	2.00	45.3†	2.05	0.49	43.8†	0.45	Rheumatic Heart Disease, Congestive Failure, Liver Cirrhosis, Cardiac
	10/ 2/35		400					4+	105	31	1.94	45.0†	1.94				

* Heparin as anticoagulant, 130 mg. per 100 cc. blood.

† Erythrocyte cell volume adjusted.

‡ Autopsy.

indicated by the cholesterol concentration. However, there was no direct quantitative correlation between the cholesterol level and the defibrinated sedimentation rate, as in apparent from the data on Cases 3 and 4, Table 18. The most striking elevations of the defibrinated rate were observed in instances of nephrosis but were not limited to this disease. A significant decrease in albumin and total protein, observed in 6 of 8 cases, was not necessarily associated with an elevated settling velocity of defibrinated blood, as also observed previously in Cases 1 and 17, Table 15.

Heparin failed to prevent clotting in 3 samples of whole blood with lipemia, as indicated in Table 18. In the remaining 5 samples the corrected sedimentation index showed linear correlation with fibrinogen although the defibrinated rate was elevated in 4 instances. The series was too small to permit further analysis.

2. *Defibrinated Blood Sedimentation Rate as a Measure of Serum Globulins and Lipoids.* All determinations of the corrected defibrinated sedimentation rate listed in Tables 15, 17 and 18 are plotted against the concentration of serum globulins in Figure 8. With the exception of instances of lipemia, the corrected defibrinated sedimentation rate shows a rough correlation with the concentration of serum globulins, as indicated by the curve drawn arbitrarily through the points. The correlation coefficient for 20 determinations shown in Table 17 was 0.74 ± 0.07 . In general this corroborates the observations of Bendien and Snapper (4). The disproportionate elevation of sedimentation rates observed in Cases 3 and 4, Table 15, may have been associated with quantitative changes of one or more components of the serum globulins, although the total concentration of these globulins was normal in each instance. It is apparent that the defibrinated sedimentation rate cannot be employed as a quantitative estimate of lipoids without further investigation and without knowledge of the concentration of serum globulins.

3. *Discussion and Summary.* The plasma fibrinogen concentration was estimated indirectly from the corrected sedimentation index of Rourke and Ernstone with an average error of 10 per cent and with extreme errors of -29 and $+41$ per cent, in blood samples which showed variable concentrations of fibrinogen and variable levels of erythrocyte cell volume but normal values for serum globulins and for

erythrocyte mean corpuscular volume. In general, this roughly quantitative correlation occurred whenever the corrected defibrinated sedimentation rate was approximately 0.1 mm. per minute or less.

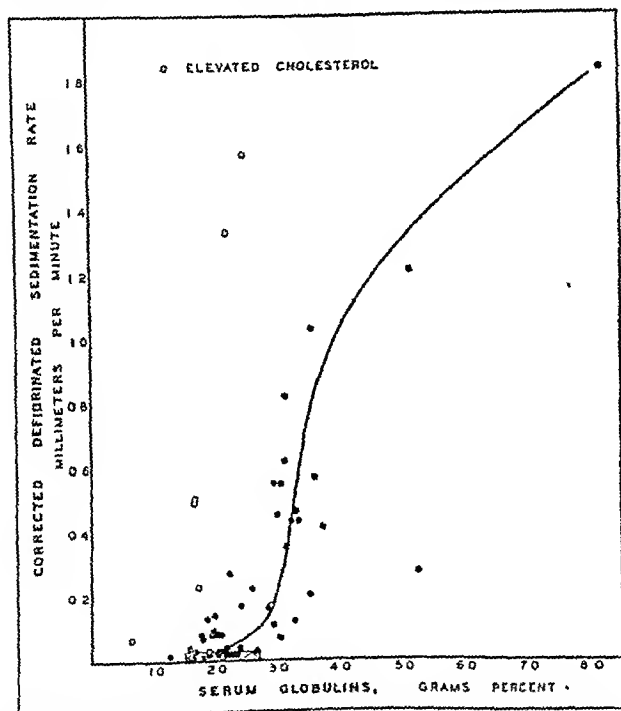


FIG. 8. CORRELATION BETWEEN SERUM GLOBULINS AND THE CORRECTED SEDIMENTATION RATE OF DEFIBRINATED BLOOD IN INSTANCES OF NORMAL AND ELEVATED CONCENTRATIONS OF SERUM GLOBULINS AND IN INSTANCES OF LIPEMIA WITH ELEVATED CHOLESTEROL.

Correlation line drawn by inspection; cross-hatched area indicates normal range

The variable factors most frequently associated with alteration of the sedimentation rate as applied clinically are the plasma fibrinogen and erythrocyte concentrations. *From a clinical viewpoint, if a blood*

sample shows a normal corrected defibrinated sedimentation rate and there is no evidence of significant macrocytosis or microcytosis, the corrected sedimentation index of whole blood by the method of Rourke and Ernstene in most instances gives a rough index of the plasma fibrinogen concentration. If the blood sample shows either an elevated corrected sedimentation rate for defibrinated blood or gross change in erythrocyte size, however, the corrected sedimentation index may fail as a rough index of the plasma fibrinogen concentration. It is also apparent that, compared to the accuracy of chemical estimation, the sedimentation rate is neither specific nor accurate as a measure of fibrinogen or any other constituent of plasma or serum.

The corrected *defibrinated* sedimentation rate in normal subjects was extremely slow and was only slightly elevated in blood samples with normal concentrations of serum globulins and of cholesterol. Although the total concentrations of serum globulins were within normal limits in these samples, it is possible that alteration of concentration of certain components of the serum globulins may be associated with greater changes in the sedimentation rate than others. With elevation of the level of serum globulins above 3 grams per cent, the defibrinated sedimentation rate was extremely variable and showed a rough correlation with concentration of globulins. In these instances the sedimentation rate of *whole blood* was markedly accelerated at plasma fibrinogen concentrations ranging from normal levels to approximately 500 mg. per 100 cc., but above this level the acceleration was less evident or absent. In subjects with lipemia, as judged from elevated plasma cholesterol concentrations, the defibrinated sedimentation rate was materially elevated in certain instances but did not correlate quantitatively with the cholesterol level. No causal relationship between the cholesterol or the lipid level and the sedimentation rate can be established from these observations. It was impossible to distinguish for any one defibrinated sedimentation rate whether an abnormal acceleration was associated with increased serum globulins or lipoids. The Takata-Ara reaction may not assist in this distinction, since it is positive in most instances of hyperglobulinemia (42, 43, 48, 58, 62, 75, 110) and may also be positive in nephrosis with normal serum globulins but elevated cholesterol (98). The Takata-Ara reaction may be positive in diabetes mellitus (110).

V. QUANTITATIVE COMPARISON OF SEDIMENTATION METHODS

As discussed in Section IV above, the technical variations in the commonly used sedimentation methods produce confusion by the difficulty of comparing results. One basis for comparison of these methods is to define for each technique its degree of fibrinogen-sedimentation correlation and the accuracy of estimating fibrinogen from the sedimentation rate under similar experimental conditions (45, 46). Accordingly, the whole blood sedimentation methods of Linzenmeier, Westergren, Cutler and Wintrobe-Landsberg were examined below and compared to the observations already described for the Rourke-Ernstene method. The influence of technical factors on the fibrinogen-sedimentation correlation was considered for each technique.

A. Wintrobe and Landsberg Method

Sixty-three determinations of the sedimentation rate, by both the method of Rourke-Ernstene and of Wintrobe-Landsberg, were made on blood samples from normal subjects and from patients who had elevated fibrinogen concentrations, as shown in Tables 13 and 15. The Wintrobe-Landsberg method was modified in one respect: Instead of a sedimentation tube of 2.5 mm. internal diameter, the Rourke-Ernstene tube of 4 mm. internal diameter was employed. Accordingly, the sedimentation rate by both methods was determined on the same tube of blood. Therefore, there were only two possible differences between these techniques: the method of *timing* and the method of *correction* for the erythrocyte concentration. The corrected sedimentation rates for the Wintrobe-Landsberg method are plotted against the fibrinogen concentrations recorded in Figure 9; the corrected sedimentation indices for the Rourke-Ernstene method are shown in Figure 6. The linear correlation of the Rourke-Ernstene corrected sedimentation index with fibrinogen is in contrast to the variable correlation of the Wintrobe-Landsberg corrected rate. For the Wintrobe-Landsberg corrected sedimentation rate the correlation with fibrinogen was roughly linear up to 400 mg. of plasma fibrinogen, but above this level there was disproportionate slowing of the sedimentation rate. The lack of a linear fibrinogen-sedimentation correlation for the Wintrobe-Landsberg method was caused in these experiments by the method of timing. As discussed previously and

as shown in Figures 1 and 2, the distance method of timing for *rapid* sedimentation rates always includes in the one hour reading the slow first period of fall and a variable portion of the third period of decreasing

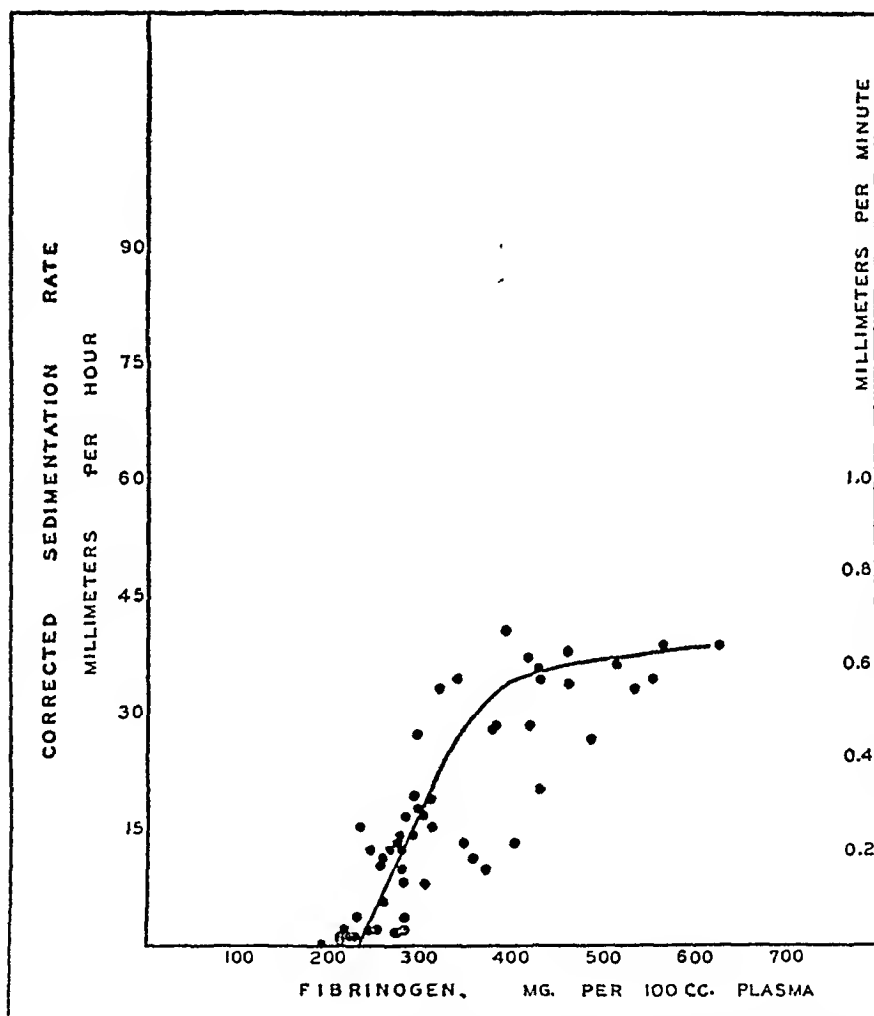


FIG. 9. CORRELATION BETWEEN PLASMA FIBRINOGEN AND THE CORRECTED SEDIMENTATION RATE BY THE WINTROBE-LANDSBERG METHOD IN INSTANCES OF VARIABLE CONCENTRATIONS OF FIBRINOGEN, BUT NORMAL CONCENTRATIONS OF SERUM GLOBULINS AND NORMAL MEAN CORPUSCULAR VOLUME.

Correlation line drawn by inspection

velocity. Therefore, due to packing, the one hour reading for rapid rates in the Wintrobe-Landsberg method will always approach a limit of 40 to 50 mm., irrespective of the sedimentation rate occurring during

the period of constant fall and irrespective of the fibrinogen content of the plasma. The distance of fall in one hour for the Wintrobe-Landsberg method may not be proportional to the settling velocity occurring in the period of constant fall.

B. Westergren Method

1. *Westergren, Theorell and Widstrom* (116) have reported the uncorrected sedimentation rates observed by the Westergren method in 100 determinations on 84 subjects, including analyses of plasma proteins and of lipoids. The statistical analysis by these authors of 62 cases showed a coefficient of correlation between the uncorrected one hour sedimentation rate and fibrinogen of 0.82 ± 0.04 . Correction for hemoglobin or erythrocyte cell volume per cent did not improve the statistical correlation.

2. *Bendien and Snapper* (4) have reported the uncorrected sedimentation rates observed by the Westergren method in 118 determinations, including analyses of plasma proteins. Since these authors used the same methods of protein analysis employed here it was possible to treat their data similarly to the data reported in Section IV. Accordingly, from the Bendien and Snapper data 61 determinations were selected in which the concentration of serum globulins was below 2.7 grams per cent. All instances of macrocytic or microcytic anemia were also eliminated. This series therefore corresponded to the classification, discussed in the section above, in which fibrinogen was elevated in concentration with normal levels of serum globulins and of erythrocyte mean corpuscular volume.

The uncorrected one hour sedimentation rates for this series has been plotted against the fibrinogen concentrations in Figure 10. There is an apparent correlation between these two variables with somewhat more spread of the determinations than was observed for the Rourke-Ernstene method on a comparable group of patients shown in Figure 6. The diagonal line represents the linear correlation as derived statistically from these data and expressed in formula (3) below. Statistically the coefficient of correlation was 0.89 ± 0.02 .

Employing formula (1) from Section IV for this series,

$$\text{Calculated } y = 6.66 (x - 28) + 507 \quad (3)$$

where (calculated y) is the fibrinogen concentration in milligrams per 100 cc. of plasma and (x) is the one hour sedimentation rate in millimeters. The fibrinogen was calculated from formula (3) for each of the 61 determinations as selected above and compared to fibrinogen level determined chemically, the error being expressed in per cent. For the whole series the average arithmetic error was 14

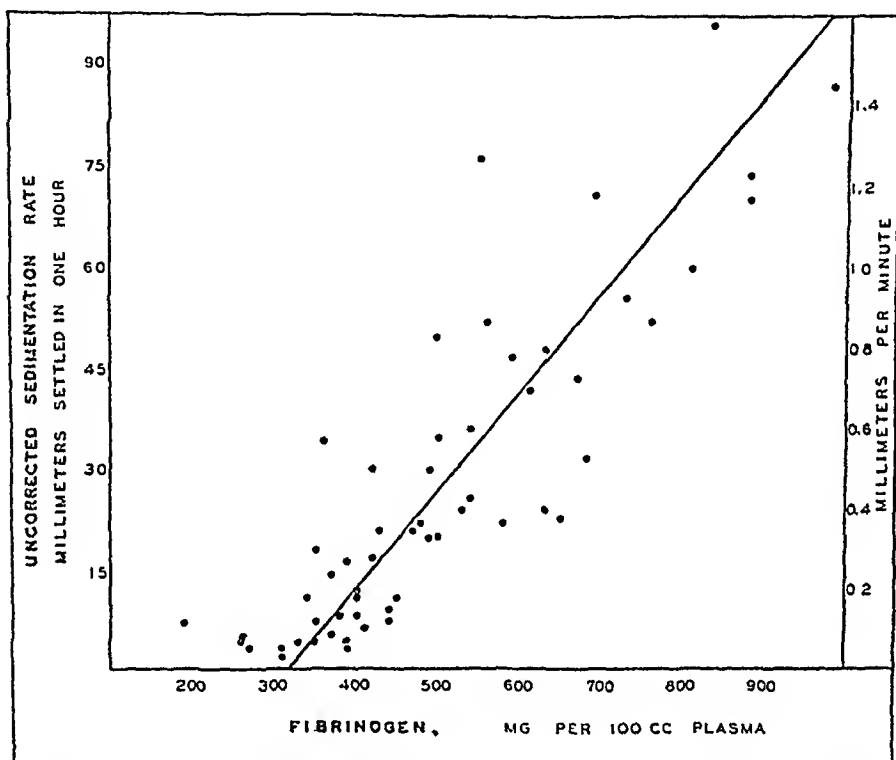


FIG. 10. CORRELATION BETWEEN PLASMA FIBRINOGEN AND THE UNCORRECTED SEDIMENTATION RATE BY THE WESTERGREN METHOD

Sixty-one determinations selected from the data of Bendien and Snapper in cases with variable concentrations of fibrinogen, but normal concentrations of serum globulins and normal erythrocyte size. Correlation line derived statistically, see formula (3) and Table 19.

per cent, with extremes of -28 and $+95$ per cent. The errors of calculating fibrinogen at three different levels of the sedimentation rate are shown in Table 19.

In the data of Bendien and Snapper for abnormally elevated concentrations of *serum globulins* (above 3 grams per cent), 31 determinations of sedimentation rates of whole blood showed a coefficient of correlation with fibrinogen of 0.4 ± 0.1 . The fibrinogen calcu-

lated from formula (3) for this series showed an average error, compared with the fibrinogen value determined chemically, of 56 per cent with a range from -14 to $+286$ per cent. The coefficient of correlation between the uncorrected *defibrinated* sedimentation rate, performed by the technique of Bendien and Snapper, and the concentration of serum globulins for 23 determinations was $r = 0.84 \pm 0.04$.

3. In the *experiments reported here*, 19 determinations of the sedimentation rate on 10 abnormal subjects were performed on samples of the same blood specimen by both the Westergren and the Rourke-Ernstene methods. The data are shown in Tables 15 and 15A. All

TABLE 19

Arithmetic Error of Calculating Plasma Fibrinogen Concentration from the Uncorrected One Hour Sedimentation Rate of Westergren in Blood Samples with Normal Levels of Serum Globulins and of Erythrocyte Cell Size

Data by Bendien and Snapper. Fibrinogen values calculated from formula (3):

$$\text{Calculated } y = 6.66 (x - 28) + 507$$

where (calculated y) is the calculated fibrinogen concentration in milligrams per 100 cc. of plasma; (x) is the uncorrected one hour sedimentation rate in millimeters.

RANGE UNCORRECTED SEDIMENTATION RATE	NUMBER OF DETERMINATIONS	ERROR IN CALCULATING FIBRINOGEN CONCENTRATION FROM SEDIMENTATION RATE	
		Range	Average
<i>mm. in 1 hr.</i>		<i>per cent</i>	<i>per cent</i>
1 to 9	19	-16 to $+95$	17
11 to 27	20	-24 to $+20$	10
30 to 113	22	-22 to $+53$	15
Total.....	61	-28 to $+95$	14

subjects had variable concentrations of fibrinogen and normal concentrations of serum globulins, but the mean corpuscular volume varied from 78 to 104 cu. microns. The erythrocyte cell volume varied from 23.5 to 49.7 per cent. The Westergren one hour reading in millimeters was uncorrected; the Rourke-Ernstene rate was corrected as usual. The fibrinogen concentration calculated from formula (3) for the *Westergren* sedimentation rates showed an average error, when compared to the fibrinogen value determined chemically, of 33 per cent with extremes of -10 and $+98$ per cent. For the *Rourke-*

Ernstene sedimentation rates on samples of the same blood specimens the average error of calculating the fibrinogen from formula (2) was 15 per cent with extremes of -21 and +38 per cent.

In the above experiments the sedimentation rate of the blood suspended in the Westergren tube was timed by both the one hour distance of fall and the slope method, as discussed in Section IV. In each instance the distance of fall in one hour obviously was less than the sedimentation rate calculated from the period of constant fall in millimeters per hour. However, the relationship between these two results was essentially linear throughout the range of sedimentation rates investigated. This linear correlation of the two results was due primarily to the extreme length of the period of constant fall for the Westergren curve, which was usually double the length of the same period observed for the Wintrobe-Landsberg or Rourke-Ernstene techniques for a sample of the same blood, as shown in Figure 1. The prolongation of this period and the delayed packing are caused by three features of the Westergren technique: the 200 millimeter height of the blood column; the slowing effect of sodium citrate solution as anticoagulant; and the narrow internal diameter of the sedimentation tube, as discussed in Section IV. As a result of these technical factors the distance method of timing for the Westergren technique usually measures comparable portions of a variety of sedimentation curves, since the one hour reading is *directly proportional* to the settling velocity during the period of constant fall.

C. Linzenmeier and Cutler Methods

In both the Linzenmeier and Cutler methods the short blood column height of 50 mm. produces a short period of constant fall and abrupt packing, as observed from the curves in Figure 1 and as demonstrated by Bannick and coworkers (1). The limitations of the timing methods have already been discussed. The variations of erythrocyte concentration influenced the sedimentation rate significantly, as shown also by Cutler and coworkers (17) and by T'ang (107). The fibrinogen concentration could not be determined from the sedimentation rate by these two methods without large error.

D. Discussion and Summary

Of the five sedimentation methods investigated under similar experimental conditions the most linear correlation between the plasma fibrinogen concentration and the sedimentation rate was observed with the Rourke-Ernstene method. In a selected series of determinations by this technique the fibrinogen concentration was calculated from the corrected sedimentation index with an average error of 10 per cent. For the Westergren method in a similarly selected series of determinations from the data of Bendien and Snapper the fibrinogen-sedimentation coefficient of correlation was slightly lower and the average error of calculating the fibrinogen concentration from the uncorrected sedimentation rate was 14 per cent. For a small series of observations on samples of the same blood specimen with variable erythrocyte cell volume and variable erythrocyte size, the average error of calculating the fibrinogen from the Rourke-Ernstene rate was 15 per cent, from the Westergren uncorrected rate, 33 per cent.

For the Rourke-Ernstene technique the erythrocyte cell volume correction method is essential to produce the above linear correlation. For the Westergren method correction is somewhat less important (1). The uncorrected one-hour sedimentation rate of Westergren produces a fibrinogen-sedimentation correlation of almost the same degree as observed with the Rourke-Ernstene procedure. Variations in erythrocyte concentration produce less alteration of the sedimentation rate for the Westergren procedure than for techniques employing anticoagulants other than sodium citrate solutions. However, on samples of the same blood specimen showing both increased fibrinogen and *anemia* the corrected sedimentation index of Rourke and Ernstene will show more linear correlation with fibrinogen than the uncorrected Westergren rate. The Rourke-Ernstene method is more reliable, therefore, as an index of fibrinogen in instances of anemia. In spite of the potential technical limitations of the Westergren method, however, its utility as an indirect measure of plasma fibrinogen is quite similar to that of the more refined and more demanding Rourke-Ernstene technique.

The methods of Linzenmeier, Cutler and Wintrobe-Landsberg produced less linear correlation between fibrinogen and the sedimentation rate than the above procedures, because of the influence of technical or erythrocyte factors, or both.

VI. CLINICAL INTERPRETATION OF THE ERYTHROCYTE SEDIMENTATION RATE

From the above experiments and from observations reported in the literature it should be possible to interpret the value and clinical significance of the erythrocyte sedimentation rate in general and the factors influencing the settling velocity in particular. There is little doubt that the sedimentation "test" has served as a valuable addition as a laboratory aid to medicine. The utility of this procedure, however, may be enhanced by a clarification of its advantages and limitations, both as a qualitative and as a quantitative test.

First, it is apparent that for *all sedimentation techniques* a normal and a pathological range can be established by clinical trial alone. This includes other "macro" methods than those discussed above (10, 112) as well as "micro" methods using capillary blood (14, 102). Employing any one of these procedures, therefore, it is possible to distinguish *qualitatively* between normal and pathological blood specimens. This information without further refinement may be of clinical value in detecting a disease condition which otherwise might escape notice.

It has been observed that the sedimentation rate is consistently *normal* during health. It may be normal also in pathological conditions which in general do not produce inflammation or active tissue destruction, such as: functional diseases, many metabolic diseases, many degenerative conditions, uncomplicated asthma and emphysema, peptic ulcer, compensated heart disease, the neuroses, nutritional deficiency diseases, and on the first day of many acute infections (24, 30, 36, 60, 76, 77, 91, 110). These conditions are usually associated with normal levels of plasma fibrinogen and serum globulins. The sedimentation rate is usually *grossly abnormal* in pathological conditions associated with inflammation and tissue destruction, as in acute and chronic infectious diseases, in local and general infections and in severe trauma. It may be *moderately abnormal* in many

instances of the following conditions: normal and abnormal pregnancy, mild infections, trauma, leukemia and lymphoma, malignant neoplasms, many liver diseases with jaundice, acute and chronic nephritis, decompensated heart disease, coronary thrombosis and thromboses in general (1, 2, 13, 21, 24, 28, 30, 41, 51, 60, 67, 74, 84, 91, 93, 120). Abnormal sedimentation rates are usually observed in diseases associated with elevated levels of plasma fibrinogen, occasionally with elevated levels of serum globulins.

As a *diagnostic procedure* the sedimentation rate has been employed to detect the presence of tissue damage or of chronic infectious disease, since it frequently is accelerated in spite of normal temperature, heart rate and leukocyte count (16, 51, 91, 117, 121). It usually is normal on the first day of an acute infection even in the presence of fever and leukocytosis, whereas it is grossly abnormal in most chronic infections (28, 51, 76, 77, 112, 121). The sedimentation rate has been used widely as a *guide to progress and treatment* of chronic infectious diseases, especially tuberculosis, rheumatic fever, arthritis and gonococcal infections (2, 21, 26, 29, 30, 73, 85, 91, 101, 114, 120). Since it frequently is the last nonspecific laboratory test to return to normal in these infections it has been a "sensitive" index of disease "activity."

The results of any method for observing the sedimentation rate thus may be correlated with health and disease and give information not obtained from other laboratory examinations. As such, the sedimentation rate may be of distinct value. However, in some instances it may give ill-defined or misleading information, as discussed below.

From a *quantitative examination* it is apparent that abnormal sedimentation rates may be correlated with abnormal concentrations of several known constituents of the plasma. This correlation occurs most frequently with *plasma fibrinogen* and in infectious disease it is so frequent that the sedimentation rate usually has served as a rough index of the fibrinogen concentration. The clinical significance of plasma fibrinogen variations in health and disease (44) has been discussed separately in the preceding paper in this Journal.

Ideally, for each method the sedimentation rate might be converted directly into the concentration of fibrinogen as done by formulas (2)

and (3) in Section IV. The advantages and limitations of the sedimentation rate as a measure of fibrinogen have already been discussed.

From the sedimentation rate of whole blood alone it may be impossible, without further examinations, to judge whether an accelerated rate is associated with an abnormal concentration of plasma fibrinogen, of serum globulins, of lipoids, of erythrocytes or of unknown factors. It may be essential to distinguish which factor is abnormal since the clinical significance of variations in serum globulins, lipoids and erythrocytes may have an entirely separate importance from that of abnormal fibrinogen concentrations. Thus in liver disease and in multiple myeloma with abnormally elevated concentrations of serum globulins, an extremely rapid whole blood sedimentation rate was observed with *normal* levels of fibrinogen in several instances shown in Table 17. Since serum globulins and lipoids are abnormally increased in concentration in a relatively limited number of diseases, such alterations have a more specific diagnostic value, and differ strikingly in their behavior from that of plasma fibrinogen in disease conditions.

To ascertain whether an accelerated sedimentation rate of whole blood correlates with an increased fibrinogen concentration it may be necessary to determine the sedimentation rate of a sample of defibrinated blood, as discussed previously. To prevent extreme errors in estimating the fibrinogen concentration it is essential in certain methods to correct the sedimentation rate for the influence of the erythrocyte concentration and to recognize the presence of grossly abnormal erythrocyte size. On the other hand, it may be simpler in some cases to determine the fibrinogen concentration directly by chemical analysis using any one of several methods (11, 33, 55, 86, 105, 123, 124).

For the estimation of plasma fibrinogen concentration several sedimentation methods, especially those of Rourke-Ernstene or Westergren, may serve as an *index* of the level which is sufficiently accurate to be of clinical value. This is especially true for obviously normal or abnormal concentrations. The accuracy may be satisfactory, also, for following the progress of subjects with chronic infections associated with grossly abnormal sedimentation rates. At

the *borderline* between normal and abnormal sedimentation values, however, other factors influencing the sedimentation rate may indicate falsely low or high fibrinogen concentrations. Accordingly, it may be *necessary* in certain instances to determine the plasma fibrinogen concentration by chemical analyses if accuracy is essential.

The sedimentation rate of *defibrinated* blood, observed either by the somewhat complex method described here or by the simple method described by Bendien and Snapper (4), may have clinical utility in detecting *qualitatively* the presence of abnormal constituents of the *serum*. Quantitatively, the defibrinated blood sedimentation rate may give a rough estimate of the concentration of serum globulins when abnormally elevated, as occurs in many chronic diseases of the liver, multiple myelomata, certain infectious diseases, including kala azar and schistosomiasis, and other diseases (9, 56, 58, 59). With increased serum globulins the Takata-Ara reaction is usually positive. An accelerated rate for defibrinated blood, however, may be associated with abnormal lipemia and normal serum globulins as observed here in nephrosis and diabetes mellitus. In the latter conditions the Takata-Ara may also be positive, as discussed above.

An abnormal sedimentation rate is always *secondary* to alterations of certain blood constituents, many of which can be defined. The interpretation of any sedimentation rate, therefore, does not depend upon the settling velocity per se but depends upon the clinical significance of those factors of plasma, serum, or erythrocytes which are abnormal.

VII. CONCLUSIONS

1. Erythrocytes suspended in plasma or serum settle because of their greater density. The wide range of sedimentation rates observed clinically results primarily from differing degrees of erythrocyte aggregation. The degree of aggregation may correlate roughly with the concentration of plasma fibrinogen, of serum globulins, or of both. The settling velocity of erythrocyte aggregates is modified by the viscosity and the limited extent of the medium.

2. The sedimentation rate is influenced significantly by variation in technique and by erythrocyte factors. An erythrocyte cell volume

correction method is described for the sedimentation rate of defibrinated blood.

3. The erythrocyte cell volume correction methods for whole blood and for defibrinated blood do not correct for the effect of variation in erythrocyte size.

4. The sedimentation rate of whole blood observed by the Rourke-Ernstene method shows a roughly linear correlation with the plasma fibrinogen concentration when there are normal levels of serum globulins, erythrocyte mean corpuscular volume and the corrected defibrinated sedimentation rate. The sedimentation rate of whole blood may fail to correlate in a linear manner with fibrinogen when there are abnormally elevated levels of serum globulins, of the corrected defibrinated sedimentation rate or advanced changes in erythrocyte size.

5. The corrected sedimentation rate of defibrinated blood normally is extremely slow as compared to that of whole blood. It may be slightly elevated in the presence of normal levels of serum globulins or of lipoids. It may be extremely elevated in instances of abnormally elevated concentrations of serum globulins and in some instances of lipemia. In the absence of lipemia there is a rough correlation between the concentration of serum globulins and the corrected sedimentation rate of defibrinated blood.

6. The sedimentation rate is not an accurate or a specific measure of any blood constituent.

7. The sedimentation rate of whole blood may serve as a rough index of the fibrinogen concentration within the limitations described above.

8. The most linear fibrinogen-sedimentation correlation was observed using the Rourke-Ernstene method. For the Westergren method the degree of correlation was slightly lower than for the Rourke-Ernstene procedure.

9. An abnormal sedimentation rate is *always* secondary to alterations of certain blood constituents, many of which can be defined. The interpretation of any sedimentation rate, therefore, does not depend upon the settling velocity per se but depends upon the clinical significance of those factors of plasma, serum, or erythrocytes which are abnormal.

VIII. APPENDIX—METHODS

A. Plasma Proteins and Cholesterol

Chemical observations were made on blood plasma, using dry potassium oxalate as anticoagulant, in a concentration of 500 mg. per 100 cc. of whole blood. The plasma fibrinogen was removed as fibrin by the clotting method of Cullen and Van Slyke (11). In instances of low or subnormal values for fibrinogen, purified cephalin (72) was added to one of the duplicates or to the fibrin-free filtrate, which was allowed to stand and examined for fibrin. This served as a control on the clot production. The albumin-globulin separation was performed by Howe's method (53,54), employing 22.5 per cent sodium sulphate. For the non-protein nitrogen separation the proteins were precipitated by the tungstic acid method of Folin (27). All nitrogen determinations were made by the micro-Kjeldahl method. Acid digestion was performed, employing the Arnold-Gunning mixture (86) and potassium persulphate as the extra-oxidizing agent (122). The acid digest was distilled by the Boch-Benedict technique (6), followed by nesslerization of the distillate and colorimetric comparison to standards containing known concentrations of ammonium sulphate. All protein determinations were done in duplicate and the results of the micro method were checked satisfactorily by the macro-Kjeldahl procedure. Plasma cholesterol was determined by a modified Bloor method (5). The Takata-Ara reaction was performed by the method as modified by Heath (48).

B. Hematology

Determinations of the red cell count, hemoglobin, white cell count and cell volume per cent were made on venous blood, using as anticoagulant either dry oxalate mixture, 200 mg. per 100 cc. of whole blood, or 20 per cent potassium oxalate solution, 200 mg. per 100 cc. of whole blood.

Dry oxalate mixture (50, 121) was made as follows: Three grams of crystalline ammonium oxalate and 2 grams of crystalline potassium oxalate were dissolved in distilled water with the aid of heat and diluted to a final volume of 100 cc. This gave an oxalate mixture concentration of 5 per cent and a 60:40 ratio of the two salts. The

employed was 200 mg. of oxalate mixture per 100 cc. of blood. Small tubes of 11 mm. internal diameter, calibrated by a diamond scratch at a volume of 0.1 cc. of blood. Then 0.08 cc. or 0.2 cc., respectively, were placed in each tube and the anticoagulant dried completely at 100°C. for one-half hour, or left for several days at room temperature. A large number of these tubes were prepared and stored.

Cell counts were made using chambers and slides calibrated by the United States Bureau of Standards. The hemoglobin was determined with a Sahli hemometer, which was calibrated by the oxygen capacity method of Van Slyke (86). One volume of hemoglobin was considered to be equivalent to 15.6 volumes of oxygen per cent, or an oxygen capacity of 21 volumes per cent. The erythrocyte cell volume per cent or hematocrit was determined by centrifugation of the blood sample at approximately 1500 rpm for 5 minutes in either Wintrobe (119) or Rourke-Ernst tubes. The blood had been allowed to stand in the tube at room temperature. When potassium oxalate solution was used as anticoagulant, the cell volume percentage was multiplied by the factor 0.92 to correct for shrinkage. No correction was necessary for dry

hemoglobin. The volume and hemoglobin content of the red blood cells were calculated by the Wintrobe method (47, 119). Thus the cell volume was determined by multiplying the cell count by 10 and dividing by the diameter of whole blood column. The mean corpuscular volume was determined by dividing the cell volume by the number of cells and multiplying by 10. The mean corpuscular hemoglobin was determined by dividing the hemoglobin content by the number of cells and multiplying by 10. The mean corpuscular hemoglobin content was determined by dividing the hemoglobin content by the number of cells and multiplying by 10. The mean corpuscular hemoglobin content was determined by dividing the hemoglobin content by the number of cells and multiplying by 10.

cal tilt-table before being introduced into sedimentation tubes. The internal diameter of each sedimentation tube was calibrated by the mercury method and tubes of uniform bore selected. The sedimentation tubes were held rigidly in a vertical position in a wooden rack especially constructed to hold a variety of tubes. The rack was enclosed in a box and the tubes viewed through a celluloid window. With the tubes in place the box was closed and the temperature maintained between 24° and 25°C. by circulating a current of conditioned air. Observations of the upper limit of the erythrocyte column as it settled from the plasma were made at intervals of from 1 to 5 minutes, depending upon the rapidity of the rate, until the period of constant fall was complete. At successive intervals of time the distance of fall was plotted graphically and the points connected. According to the slope method of timing described in detail by Rourke and Ernstene (94), the straight line portion of the period of constant fall was extended to the base line and to any selected point above the base line. The ordinate of a selected point on this line, in millimeters, was then divided by its abscissa, in minutes. The sedimentation rate was reported in millimeters per minute or per hour. The timing method required by each author's method was also employed.

1. *Rourke-Ernstene* (94). For the Rourke-Ernstene method venous blood was drawn as above, employing as anticoagulant either dry oxalate mixture, 200 mg., or heparin in a concentration of 65 mg. or 130 mg. per 100 cc. of mixture, as indicated in the tables. Heparin was prepared in a 15 per cent solution in distilled water, using the product of Hynson, Westcott and Dunning; 5 different lots were employed. The Rourke-Ernstene tubes were made with the following specifications: length 12 cm., internal diameter 4 mm. (calibrated), pyrex glass, calibrated for 5 mm. above the zero mark and every millimeter for 100 mm. below the zero mark, Macalaster Bicknell, Cambridge. The sedimentation rate was observed as described above. The hematocrit was observed by centrifuging the sedimentation tube as above. The uncorrected sedimentation rate was corrected according to the hematocrit to an erythrocyte cell volume of 45 per cent, employing the correction chart devised by Rourke and Ernstene, and the rate reported as the "corrected sedimentation index" in millimeters per minute.

2. *Linzenmeier* (69). For the Linzenmeier method, 1.5 cc. of venous blood was introduced into a calibrated collecting tube containing 0.4 cc. of 5 per cent sodium citrate solution, the sample mixed and pipetted into the Linzenmeier tube, made according to the following specifications: internal diameter 5 mm., length 7 cm., blood column height 50 mm., calibrations in millimeters. The time in minutes required for the red cells to fall to the 18 mm. mark was recorded. There is no correction for the concentration of erythrocytes.

3. *Westergren* (113, 115). In the Westergren method, 1.6 cc. of venous blood was introduced into a calibrated collecting tube containing 0.4 cc. of 3.8 per cent sodium citrate solution. The sample was mixed and drawn into the Westergren tube: internal diameter 2.5 mm., length 30 cm., blood column height 200 mm., calibrated in millimeters. The distance of fall of the erythrocyte column was recorded in millimeters at the end of one hour without correction for the erythrocyte concentration.

4. *Cutler* (15). For the Cutler method, 1.8 cc. of venous blood was introduced into a calibrated collecting tube containing 0.2 cc. of 3 per cent sodium citrate solution. The sample was mixed and pipetted into the Cutler tube: 5 mm. internal diameter, 7 cm. length, 50 mm. blood column height, calibrated in millimeters. The sedimentation rate was measured by the slope method and by the distance of fall in one hour, measured in millimeters.

5. *Wintrobe-Landsberg* (121). For the Wintrobe-Landsberg method, blood was collected as for the Rourke-Ernstene technique, mixed and pipetted into a *Rourke-Ernstene* sedimentation tube. This was a modification of the Wintrobe-Landsberg method only insofar as the tube employed. The Wintrobe tube has an internal diameter of 2.5 mm., a length of 12 cm., a blood column height of 100 mm. and is calibrated in millimeters. The hematocrit was determined as above. The one hour distance of fall in millimeters was recorded and corrected for the hematocrit level by the Wintrobe-Landsberg correction chart to a cell volume of 42 per cent for females and 47 per cent for males.

D. Defibrinated Blood Sedimentation

The sedimentation rate of *defibrinated blood* was determined by a technique which was a modification of the method of Bendien and

Snapper (4) Five cubic centimeters of venous blood were introduced into a 25 cc Erlenmeyer flask containing 8 to 12 glass beads of 2 mm. diameter. The flask was stoppered and gently rotated for 10 to 15 minutes, or until the beads were seen to be covered with fibrin. The blood was then poured through a small funnel containing a plug made from two layers of clean gauze, about 1 cm square, in order to catch the beads. The defibrinated blood was mixed, pipetted into a Rourke-Ernstene tube and the sedimentation rate observed as with whole blood. Because of the extremely slow settling velocity of many samples, readings were required at 10-minute intervals for from 1 to 3 hours. The erythrocyte cell volume was always found to be 1 or 2 per cent higher than the corresponding whole blood hematocrit. A slight amount of hemolysis was usually present. The sedimentation rate in millimeters per minute was observed by the slope method and corrected according to the hematocrit to an erythrocyte cell volume of 45 per cent, employing the correction chart described here (Figure 4) or, for more rapid rates, the Rourke-Ernstene graph for whole blood (94).

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